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RELATIVE GROWTH IN THE HOUSE WREN*

By

SARA E. HUGGINS

Biological Laboratory, Western Reserve University

(*Received for publication June 7, 1940*)

Most relative growth studies have of necessity been based on the assumption that the results are the same whether each age or size group is represented by a different set of individuals or whether the same group is used at each stage. It is possible that the individuals represented in the average may vary materially. The study of the individual growth pattern is therefore an interesting check on generalizations made concerning the growth pattern of the species. Difficulties of rearing often stand in the way of the investigator's use of the same individuals day after day. However, because of their nesting habits, live house wren material is readily available. Under the unique facilities of the Baldwin Bird Research Laboratory it was also possible to have a large number of nest boxes under observation at one time. Long experience by associates of this laboratory indicates that when reasonable care is exercised the adult birds will carry on the feeding and other nest duties regardless of human intrusion.

There have been a few studies which dealt with individuals such as those of Anderson (1932) on *Daphnia magna* and Hersh (1938) and Clark and Hersh (1939) on *Notonecta undulata*.

MATERIALS AND METHODS

The data incorporated in this study were obtained from ninety-two nestling house wrens (*Troglodytes domesticus baldwinii Oberholser*) and six juvenile and ten adult birds of the same species. Measurements were taken with a pair of needle pointed dividers and recorded to the nearest half millimeter. The measurements made included total length without feathers, total length of culmen, length, width, and height of head, length of neck, body and tail, width of body, length of femur, tibio-tarsus, tarsometatarsus, middle toe, middle nail, hind toe, hind nail, upper-arm, fore-arm, and manus. Most of these were made as Baldwin, Oberholser, and Worley (1931) direct. But, because it was desirable, for this study, to have a consecutive series of body segments which did not overlap, the neck length is that from the base of the cranium to the anterior angle of the shoulder, holding the neck fully extended. The body was considered to extend from this same angle of the shoulder to the base of the tail. Since it was impractical to make

*I wish to acknowledge the great privilege given me by the late S. Prentiss Baldwin in the use of the facilities of his Bird Research Laboratory.

TABLE I

AVERAGE DIMENSIONS[1] IN MM., OF THE DIFFERENT ORGANS OF THE HOUSE WREN, FOR THE FIRST 16 DAYS AFTER HATCHING

Days	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Juvenile	Adult
Bill	3.28	3.81	4.50	5.28	6.01	6.59	7.57	7.93	8.95	9.36	9.95	10.25	10.67	10.90	11.06	11.32	12.12	13.42	14.80
Head Length	9.93	10.56	11.45	12.55	13.38	14.41	15.00	15.66	16.18	16.56	16.62	16.90	17.25	17.32	17.50	17.60	17.38	17.30	17.25
Head Width	7.62	8.28	8.92	9.73	10.32	11.00	11.52	11.95	12.54	12.79	13.14	13.35	13.46	13.50	13.62	13.82	13.75	13.62	14.57
Head Depth	6.71	7.46	8.37	9.12	9.55	10.36	10.80	11.28	11.55	11.69	12.09	12.01	12.00	12.22	12.21	12.22	12.50	12.33	13.12
Femur	6.66	7.98	9.04	10.71	12.47	13.78	14.85	15.40	16.02	16.21	16.71	16.71	17.02	17.14	17.23	17.18	17.50	15.33	17.58
Tibiotarsus	8.71	10.25	12.27	14.66	16.78	18.83	20.82	22.02	23.35	23.94	24.48	24.76	24.78	24.98	24.96	24.96	25.00	25.00	25.62
Tarsometatarsus	5.41	6.44	7.68	9.25	10.88	12.75	14.20	15.21	16.14	16.68	17.14	17.28	17.52	17.48	17.52	17.54	17.00	17.00	17.38
Middle Toe	4.46	5.15	6.12	7.71	9.07	10.41	11.45	12.09	12.48	12.48	13.00	12.93	13.30	13.17	13.13	12.96	12.50	13.25	12.88
Middle Nail	.60	.62	.95	1.18	1.53	1.80	2.09	2.48	2.50	2.77	2.82	3.04	3.22	3.30	3.31	3.54	3.00	4.12	3.57
Hind Toe	3.03	3.54	4.15	5.02	5.95	6.72	7.47	7.78	8.27	8.52	8.55	8.60	8.70	8.76	8.81	8.64	8.00	8.62	8.19
Hind Nail	1.00	1.07	1.54	1.76	2.16	2.35	2.86	3.20	3.40	3.68	4.04	4.16	4.48	4.53	4.65	4.68	4.50	5.75	5.78
Neck	5.94	6.77	7.81	8.52	9.46	10.25	10.95	11.15	11.54	12.29	12.68	12.96	13.28	13.54	13.62	13.73	11.50	12.50	14.57
Body	13.86	15.45	17.77	20.24	22.26	24.58	26.00	27.05	28.13	28.45	29.36	29.54	30.08	30.43	30.41	30.64	31.00	32.62	31.71
Tail	1.92	2.14	2.32	2.80	2.98	3.17	3.78	3.92	3.96	4.23	4.46	4.42	4.45	4.46	4.50	4.50	4.25	4.38	5.43
Upper-arm	5.46	6.44	7.43	9.04	10.13	11.50	12.55	13.40	14.84	15.16	15.93	16.03	16.12	16.52	16.54	16.82	17.00	16.17	16.58
Fore-arm	4.90	5.52	6.40	7.71	9.05	10.72	11.85	12.83	14.04	14.60	15.36	15.61	15.82	16.12	16.22	16.46	16.25	16.50	16.78
Manus	5.59	5.94	6.95	8.41	9.93	11.14	11.98	12.55	13.50	13.58	14.30	14.32	14.58	14.69	14.90	14.93	15.00	14.75	15.05
Width of Abdomen	7.74	9.02	10.69	11.52	12.14	12.75	13.04	13.38	13.35	13.25	13.27	13.06	13.08	12.88	12.92	12.69	12.00	15.25	13.00
Total Length	32.09	35.54	40.55	46.43	51.27	55.48	58.90	61.31	67.12	68.26	70.18	71.12	72.25	73.12	73.21	75.25	71.83	73.00	84.17
Weight		1.98	2.52	3.75	5.17	6.60	7.82	9.08	9.42	9.93	10.47	9.82	10.52	9.88	9.92				

[1]Each average dimension represents about thirty cases except those for the fifteenth day for which only 15 individuals were available, the sixteenth day and juveniles with only about five each, and the adults with about ten. Tables giving exact numbers for each day and each measurement, as well as all individual records and relative growth constants for individuals are available in thesis form in the Western Reserve University Library.

many additional measurements on the same nestlings, weights and feather lengths used for purposes of comparison were obtained from the unpublished notes of the Baldwin Bird Research Laboratory.

The data have been analysed, using the relative growth equation $y = bx^{\alpha}$. In this equation y represents a dimension of one part and x equals a dimension of the whole or of another part, b is the index of initial size, and α is the equilibrium constant. This method of analysis may be used advantageously to bring out such growth gradients as may be present, to show shifts in growth centers, and to emphasize the resulting changes in form. Values of α, b, and P. $E._{\alpha}$ were calculated as suggested by Feldstein and Hersh (1935).

All available material was analysed statistically to determine in so far as possible whether or not handling, ecto-parasites, or size or season of brood have any effect on the growth rate of nestlings. Handling apparently has little or no effect on the growth rate. Lice seem to have a slight detrimental effect. Birds from smaller broods seem to be slightly larger except in a few cases of very small broods. As second broods are usually smaller this advantage holds for birds hatched later in the season.

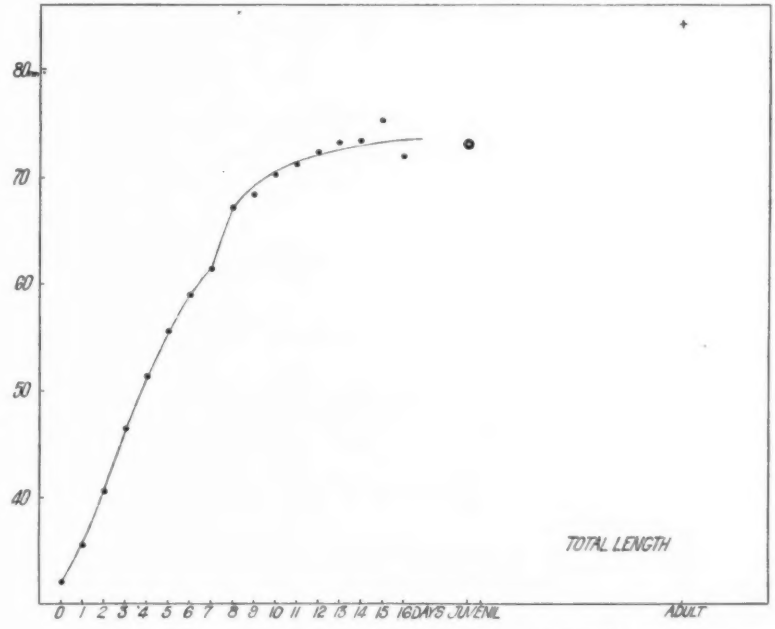

Fig. 1. Time course of increase in total length.

RESULTS

The time course of the various measurements approximate sigmoid curves, the period of most rapid growth being between the second and the seventh or ninth days. The lower flattened portion of the curve is shorter than the upper in most cases (Figs. 1-3).

Double log graphs of these measurements plotted against total length seem to be well fitted by one or two straight lines. Where two are used

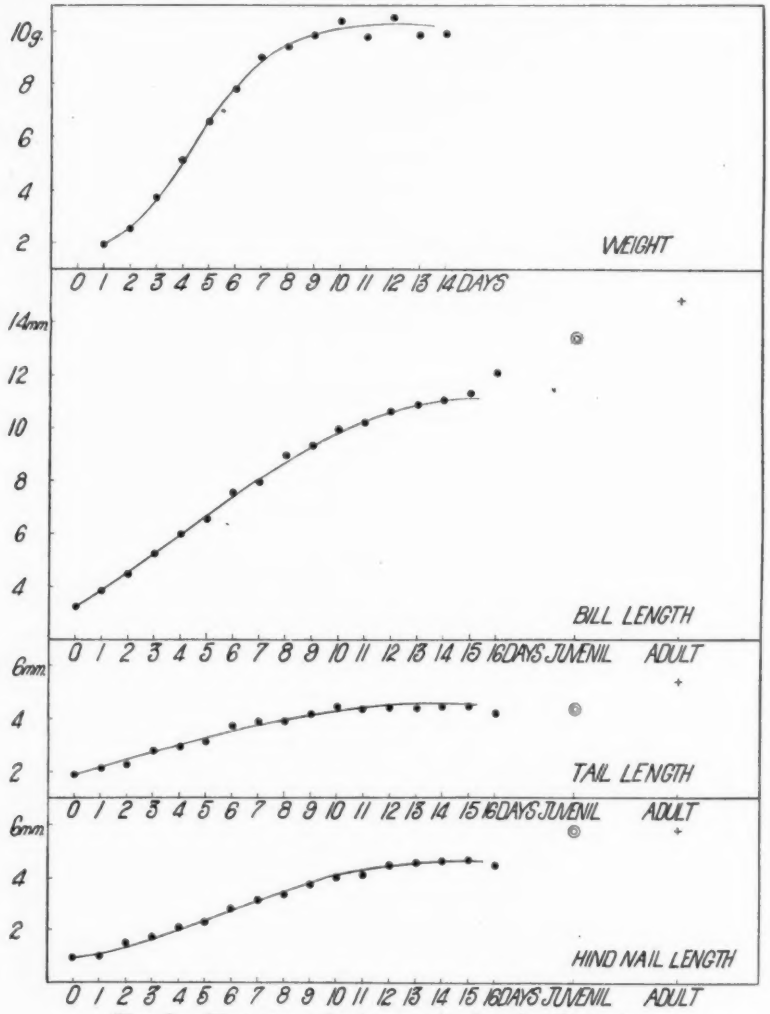

Fig. 2. Time course for increase in size of various parts.

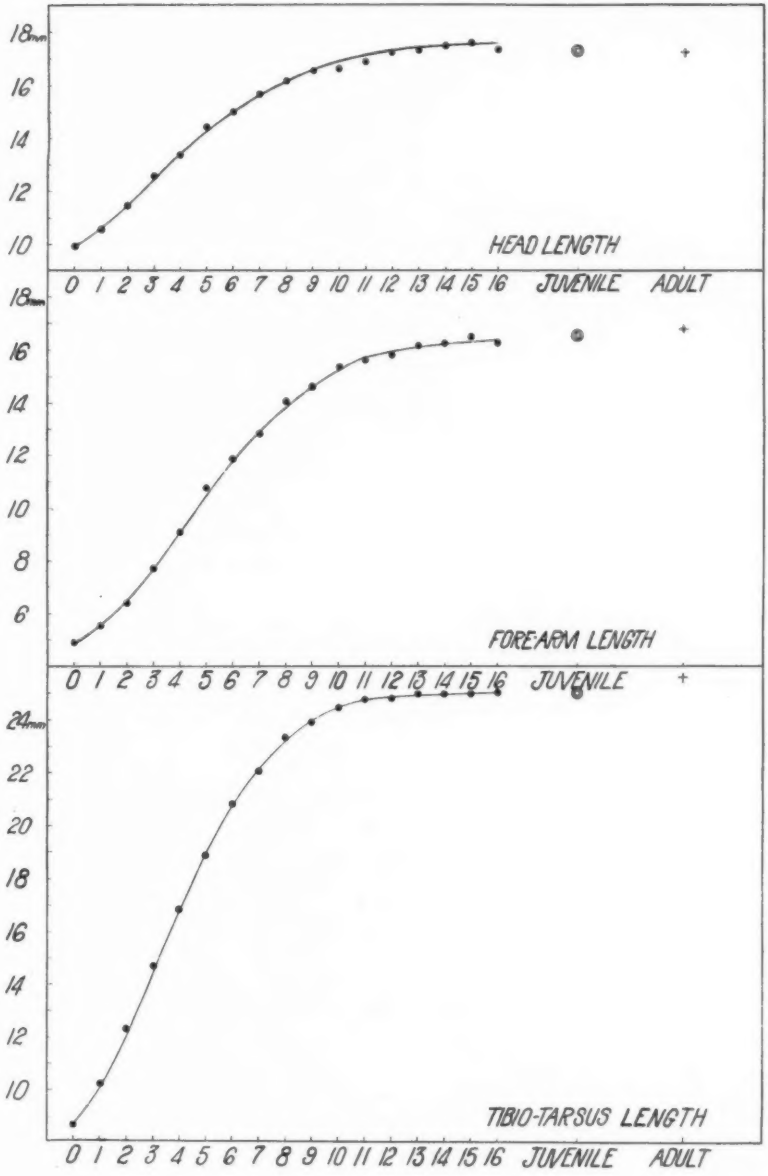

Fig. 3. Time course for increase in size of various parts.

the break occurs between the seventh and eighth days. This break in the relative growth curve occurs as the variables approach their asymptotes. Lumer (1937) has demonstrated that if the time course of growth of both y and x is sigmoid, conforming to one of several growth formulae, for example the simple autocatalytic, then the relative growth function gives a first approximation for the relation between y and x but is not valid over the entire growth period. As the dimensions approach their upper asymptotes the straight line relationship breaks down and as the coefficient of growth partition between the parts may make a number of successive changes rapidly a curve rather than a straight line is generated. For this reason the constants of relative growth beyond the eighth day have no particular significance although they have been calculated. Figures 4-8 show the relative growth curves.

The values of the relative growth constants for average measurements are given in Table II. It will be observed that a postero-anterior gradient exists along the main body axis prior to the eighth day. The bill is an exception to this gradient but this seems only natural since it undoubtedly arises late in development.

There is a disto-proximal gradient in the leg segments with only a slight irregularity between tarsometatarsus and middle toe. The gradient is proximo-distal beyond the eighth day. This is only accidental as will be shown by the results from a study of individuals beyond the eighth day. The nails continue to grow at a high rate throughout the nestling period.

The wing does not have a regular gradient. The high point of growth is in the fore-arm. The upper-arm has the next highest rate. This indicates a concentration of growth partition proximally. The wing segments do not show any apparent break on the eighth day.

Of the measurements taken the width of the abdomen is the only one which actually decreased with time. This is probably related to the larger proportional food intake of the younger birds. The measurement of the depth of body at the posterior end of the sternum, made for a few individuals, showed this same decrease.

That there is little change in head shape during the nestling period is indicated by the values of α for the three head dimensions, length, width, and depth. Things growing at the same rate will have similar equilibrium constants as these do.

The weight compared with total length has an α value of 2.86 which is not far short of the theoretical value, three.

Values of the constant b show interesting correlations with α in some cases. For example, the values of b for the leg before the eighth day form a perfect series being large for the femur and becoming progressively smaller distally. This series is the reverse of the α gradient and

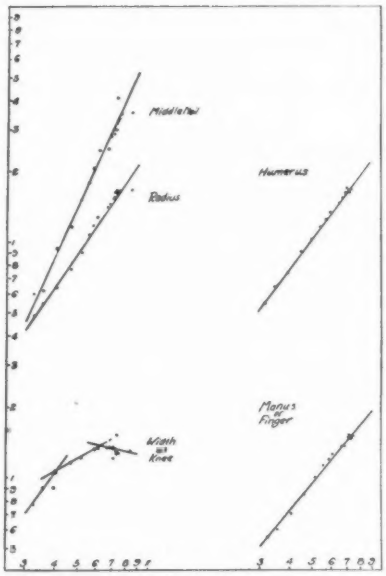

Fig. 4

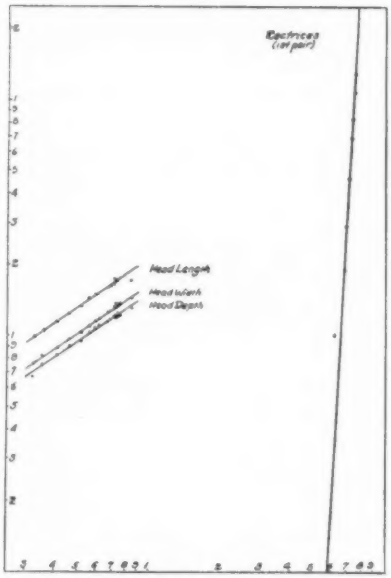

Fig. 5

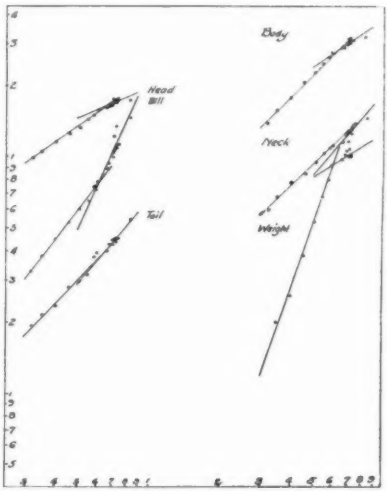

Fig. 6

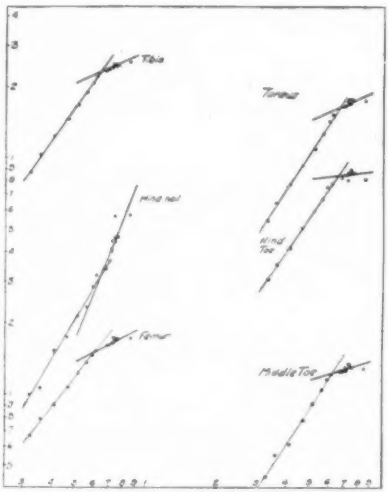

Fig. 7

Log-log graphs of various regions plotted against total length.

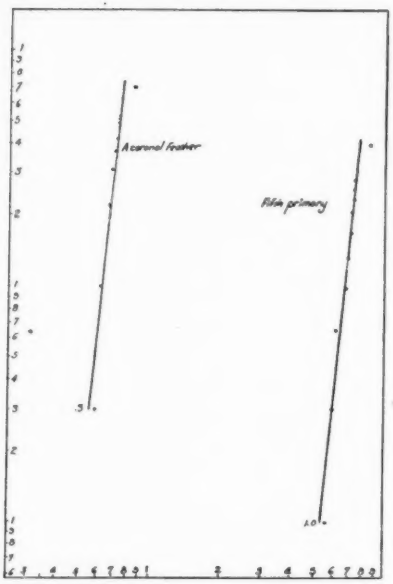

Fig. 8. Relative growth curves, each compared with total length.

is more regular, lacking the discrepancy between tarsometatarsus and toe. In the case of the wing the values of *b* are inversely proportional to the α values. This relationship does not hold for the body segments but the bill which has a very high value for α does have a very low one for *b*.

The relative growth constants for individuals differ from the average, yet the gradients may still be similar. In 17 out of 20 individuals the disto-proximal gradient of the leg of the average is approximated although the α values vary considerably from the average.

The values of α for leg segments beyond the eighth day were calculated for a number of individuals. They do not conform to the proximo-distal gradient found for the average but are distributed as if by chance.

The gradient along the main body axis for individuals is less constant than that of the leg. The segment showing the most variation is the neck.

The results obtained in calculating the constants for the individual birds seem to sustain the conclusions drawn from the average results in some instances. The gradient of the leg before the eighth day seems especially constant and therefore to justify the generalizations made. The gradient along the main body axis is less constant but is probably

TABLE II
TABLE OF CONSTANTS FOR AVERAGES

	0-8 days			8-adult		
	a	PE_a	b	a	PE_a	b
Bill	1.3205	±0.0081	0.03373	2.2516	±0.0395	0.00014
Head Length	0.6988	±0.0042	0.86840	0.4193	±0.0173	2.81066
(all)	0.6749	±0.0025	0.94508			
Head Width (all)	0.6976	±0.0012	0.67560			
Head Depth (all)	0.6713	±0.0276	0.68628			
Neck	0.9599	±0.0034	0.21745	1.1620	±0.0293	0.08878
Body	1.0187	±0.0015	0.40700	0.6740	±0.0167	1.63190
Tail	1.0551	±0.0086	0.04861	1.1781	±0.0238	0.28570
(all)	1.0577	±0.0070	0.04796			
Upper-arm (all)	1.3270	±0.0029	0.05512			
Fore-arm (all)	1.4914	±0.0044	0.02651			
Manus (all)	1.2150	±0.0041	0.08044			
Thigh	1.2863	±0.0030	0.07812	0.5353	±0.0174	1.69250
Tibiotarsus	1.4106	±0.0043	0.06577	0.4546	±0.0115	3.52300
Tarsometatarsus	1.5778	±0.0043	0.02250	0.4487	±0.0179	2.50800
Middle Toe	1.5688	±0.0029	0.01893	0.2890	±0.0180	3.74350
Middle Nail	2.2190	±0.0034	0.00024			
Hind Toe	1.4712	±0.0031	0.01822	0.1104	±0.0204	5.30325
Hind Nail	1.7540	±0.0117	0.00219	2.6353	±0.0511	0.00006
Width-Abdomen (0-2)	1.3783	±0.0054	0.06472	—0.2181	±0.0223	33.44500
(3-7)	0.5352	±0.0021	1.47800			
Weight	2.8582	±0.0253	0.00007	0.5350	±0.0332	1.04330

real. That there is probably no significance in the apparent reversal of the leg gradient after the eighth day, is shown by the great variation found among individuals as well as the limitations of the relative growth formula pointed out before.

DISCUSSION

The postero-anterior gradient found along the body axis and the disto-proximal gradient of the limbs are the reverse of the gradient found among most animals during embryonic development. The concept of the head as the center of greatest growth and highest metabolism is very familiar. If, however, these patterns remained the same throughout life the adult proportions could not be attained and certain functions, for example flight and bipedal locomotion, would be impossible. Therefore, the reversal or at least alteration in gradient is to be expected. Certain structures, such as the long curved nails and long slender bill of the adult wren might be disadvantageous to the bird while in the egg, yet must reach useful proportions rapidly during the brief nest life, and so must have very high relative rates of growth after hatching. This of course is true of any structure arising late in embryonic development or, as in the case of the feathers, after hatching.

Some of the data in the literature furnishes interesting comparisons for the data on the growth of the hind limb of the wren. This is especially true of the data on the mouse presented by Green and Fekete (1933). The values of α for femur, tibia, and metatarsus form the same gradient found for the wren and are similar in values. The values of b for the first two segments are almost identical although those for the metatarsus are quite different.

The principal relative growth studies on birds made previously are those of Schmalhausen (1927a, 1927b, and 1927c) and Lerner (1936, 1938a, and 1938b). The chief animal used by both investigators being the domestic chicken.

The values of α given by Lerner were calculated by comparing length of leg bones to weight. If weight is considered the cube of length and for purposes of comparison the α values are multiplied by 3, the growth rates of femur and tibia are very similar to those of the mouse, that is slightly lower than those of the wren. The gradient is the same for most breeds and cross breeds of chicken used though the value of α for the metatarsus is considerably smaller than for either the wren or the mouse. This seems to indicate that there may be a common growth pattern among the higher vertebrates in the case of the hind limb.

Green and Fekete found a similarity between the gradients in fore and hind limbs of the mouse but this is not true of the wren. There is little material from which to draw comparisons. However, the data of Beebe and Hartley (1917), Parker (1891), and Marples (1930) may be analysed so as to give comparative rates of growth for the three wing segments although the material published is not of a character that makes calculation of the constants possible. In 16 of the 20 species the fore-arm has the highest rate of growth. This is also true of the wren. In the remaining four cases the high point is in the hand. Thirteen species have the lowest rate of growth in the upper-arm and seven in the hand. Beebe and Hartley indicated that they believed there was a correlation between flight habits and growth pattern. The data of Parker on several species of Apteryx are interesting in that the rate of growth of the upper-arm is much nearer the rate of the fore-arm than in any of the other species and that of the hand very much lower.

SUMMARY

The time course of growth for all measurements made was sigmoid with the period of greatest growth between the second and seventh or ninth days.

The application of the relative growth equation $y = bx^{\alpha}$ showed that the wren has a postero-anterior gradient along the main body axis up to the eighth day with values of α as follows:—bill, 1.320; head, 0.699; neck, 0.960; body, 1.019; tail, 1.055.

The gradient found along the leg for the average wren has values of α as follows:—femur, 1.286; tibio-tarsus, 1.411; tarsometatarsus, 1.578; middle toe, 1.569; nail, 2.219. This gradient is also found among other higher vertebrates.

The growth pattern in the wing is irregular. The values of α are as follows:—upper-arm, 1.327; fore-arm, 1.491; manus, 1.215. This pattern does not conform to that found among mammals but is common among birds.

Little significance is attached to the constants calculated beyond the eighth day. There are two reasons for this, first the inadequacy of the relative growth formula for periods when the time course of growth is approaching its asymptote and second the great variation found in the constants and gradients of individuals for this period.

Constancy of form necessitates similarity in equilibrium constants, as for example those for the dimensions of the head of the wren.

Individuals may vary from the average with respect to growth gradients, as shown by the order of relative growth intensities. The constants representing these intensities may also vary considerably. The extent of variation among individuals may determine the validity of assumptions based on the average.

I wish to express my appreciation to Dr. A. H. Hersh, Dr. B. C. Anderson, and Dr. S. C. Kendeigh for their many helpful suggestions.

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CORRELATED GROWTH IN THE LEAF OF BEGONIA ARGENTEO-GUTTATA

By

JAMES NEEL

Department of Biology, Dartmouth College

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Scattered over the upper surface of the leaves of *Begonia argenteo-guttata* are numerous small, irregularly ovate, silvery spots. These range in size from flecks consisting of a few cells up to areas six to seven square millimeters in extent, involving 2500-3000 epidermal cells. Hassack (1886) sectioned the leaves of various species of silvery-spotted Begonias and found that the spotted areas were characterized by a slight and irregular separation of the upper epidermis from the underlying palisade parenchyma. The small cavities formed as a result of this separation were thought to be filled with air. It was suggested that where these cavities were present in the leaf, there would be differences in the manner in which incident light was reflected, and these differences might well make the distinction between green and silvery color.

In the approximate center of some of the smaller and almost all of the larger of the spots of *Begonia argenteo-guttata*, a single "hair" is found. This hair is a multicellular outgrowth of the epidermis; it is usually quite transparent but sometimes shows a pink core at the base. Hairs have never been observed on the non-spotted portions of the upper surface of the leaf.

Casual inspection suggested that there is a relation between the area covered by a spot and the length of the hair associated with it. Accordingly, 130 spots and the corresponding hairs (when one was present) were drawn with the aid of a camera lucida. The length of each hair was determined by rolling a small calibrated wheel along the center of the drawing of the hair and making the appropriate reduction in the length so obtained. To establish the area of a spot, the size of the spot in the camera lucida drawing was determined with a planimeter, and the necessary reduction made. The leaves studied were freshly but fully expanded and all came from the same plant.

In Figure 1 the length of the hair, when one is present, is plotted against the area of the spot. A number of interesting relationships are apparent. These may be considered in terms of (a) distribution of hairs and (b) size of hairs.

(a) *Distribution of hairs.*

Only occasionally (5 out of 25 cases) are well developed hairs found on spots whose area is less than 0.5 mm.^{2} In the majority

of such small spots either there is no trace of a hair, or only a very small stub, whose length is less than 0.1 mm. In slightly larger spots, ranging in size from 0.5 to 1.2 mm.^{2}, the situation is quite different, since now the majority of the spots (34 out of 43) possess hairs. Finally, only 3 out of 62 spots whose areas are greater than 1.2 mm.^{2} are without hairs. As a general rule, then, hairs are found associated with spots only when a certain minimal size has been exceeded; spots which have exceeded that size seldom lack hairs.

(b) *Size of hairs.*

Only two hairs have been observed whose length is less than 0.8 mm. Either a spot has no hair or a very small stub, or the hair exceeds 0.8 mm. in length. In this respect the appearance of a hair partakes of the nature of an "all-or-none" reaction: once a certain area, which varies somewhat from spot to spot, has been reached, a well-formed hair appears.

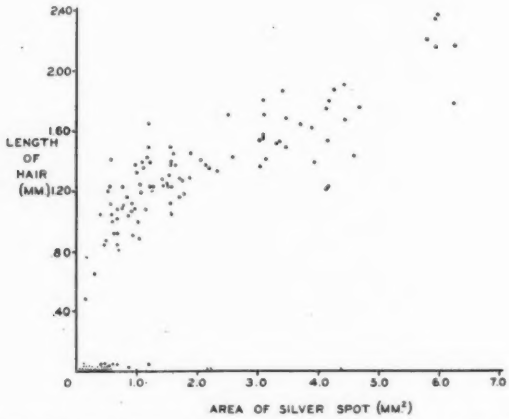

Fig. 1. The correlation between the area of a silvery spot and the length of the hair which occurs on it, in leaves of *Begonia argenteo-guttata*. Those spots which do not possess hairs are represented by (x), while spots with either a very small stub or a well developed hair are shown by dots.

In spots which do possess hairs, there is a simple relation between the area of the spot and the length of the hair. Hairs associated with large spots are regularly longer than those associated with small spots. This is apparent from the figure. For all spots which show well developed hairs (98), the correlation between spot area and hair length is 0.753 ± 0.044. At least two factors operate to make this observed correlation somewhat less than the actual value: (a) In the determination

of the areas of spots and lengths of hairs, errors creep in, through the use of a camera lucida and a planimeter. In the case of small spots, it is estimated that the error in area may in some cases amount to 10 per cent. The errors in hair length are probably somewhat less. (b) Occasionally a small black tip was noticed on a hair. This tip is due to a few black and shriveled cells. The occurrence of these slightly necrotic hairs introduces a source of variation into the size-area relationship which is not inherent in the growth mechanism.

In order to determine more exactly just what is involved in the hair-spot correlation, Begonia material was fixed and sectioned. In cross sections of leaves, spotted areas could be distinguished from non-spotted by the occurrence in the former of numerous small spaces between the epidermis and palisade parenchyma; such spaces were lacking in the latter. When pieces of fresh Begonia leaf are mounted in water under a cover slip, frequently a stream of small bubbles is seen to issue from a spot. This suggests that in life the spaces are filled with air, or that a spot represents a more favorable place than the surrounding tissue for the escape of the air contained in the leaf. The spots tend to fade when the leaves are soaked in water, probably due to the penetration of water into the air spaces. These findings are in agreement with the above-cited work of Hassack (1886). Coupled with the occurrence of these spaces was a lessened regularity in the arrangement of the cells of the underlying palisade parenchyma. The epidermal cells involved in a spot were somewhat smaller than those in non-spotted areas. Perhaps as a result of this smaller cell size, the epidermis of a spot had a slight tendency to be depressed below the surrounding epidermis. No other distinctions between the silvery and green appearing portions of the leaf were noted. Actually, then, our growth correlation is between the length of a hair and the extent of the area characterized by small epidermal cells, a slight separation of these from the underlying parenchyma, and some irregulariy in the distribution of the palisade parenchyma.

The agent which correlates spot and hair growth is as yet unknown. The simplest assumption would be that spot and hair are derived from the same anlage, and that from the moment of their determination the cells of the developing hair and spot retain the same relative rates of multiplication.

The restriction of hairs to spots, the "all-or-none" nature of hair occurrence, and the correlation between hair and spot size—all combined in a relatively simple system—make this material unusually favorable for a study of determination and correlated growth in plants.

SUMMARY

The upper surface of the leaf of *Begonia argenteo-guttata* is covered with silvery spots. Epidermal hairs, when present on the upper surface of the leaf, are restricted to the approximate center of these spots, one hair to a spot. Hairs are not often found in connection with spots whose area is less than 0.5 mm.^{2} When hairs are present, there is a high correlation between the area of a spot and the length of the hair associated with it.

I am indebted to Dr. Hannah Croasdale for technical assistance.

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HETEROGONY IN CELL HIERARCHIES

By

W. F. FLOYD

Middlesex Hospital Medical School, University of London, England

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The terminology employed in this paper is based on that of Woodger (1937). We consider cell-hierarchies which begin with a zygote, are $1 \rightarrow 2$ and regular in Woodger's sense, i. e. for any n, if the nth level exists it has 2^n members. But we introduce two new parameters namely, that of the time-length or duration of a cell and that of volume-ratio (to be defined later) of a cell. When such a cell-hierarchy contains sub-hierarchies in which these parameters are different from those for the remaining cells, we show that Huxley's heterogonic growth law (1924, 1932) can be derived as a consequence of the primitive assumptions made about cell-growth with respect to volume. It is not claimed that what follows is by any means a complete description: rather it indicates what can be accomplished by the treatment of but one or two primitive concepts.

Let x be a single cell in time extension. By 'the time-length of x' (Tl'x) is meant the stretch of time from the initial state of x (denoted by B'x) to the final state of x (denoted by E'x). The initial state of x is the momentary state immediately after the formation of x by division (or by fertilization in the case of a zygote), and the final state of x is the momentary state immediately before the division of x into two cells. Vol'B'x will denote the volume of the initial, and Vol'E'x the volume of the final, state of x.

Let x divide into two cells y and z. Then

$$\text{Vol'E'x} = \text{Vol'B'y} + \text{Vol'B'z} \qquad (1)$$

expresses the assumption that there is conservation of volume at division. Also let

$$\text{Vol'B'y} = \text{Vol'B'z} \qquad (2)$$

By the volume-ratio of a cell x we shall mean half the ratio of Vol'E'x to Vol'B'x and we shall denote it by Vr'x. If

$$\frac{\text{Vol'E'x}}{\text{Vol'B'x}} = 2\alpha$$

then with assumptions (1) and (2) we shall have

$$\text{Vol'B'y} = \alpha.\text{Vol'B'x}$$

Now consider a cell-hierarchy which begins with a zygote, is regular and $1 \rightarrow 2$. We shall say that such a cell-hierarchy is regular with

respect to time-length and volume ratio up to the nth level (more briefly T,α,n-regular) if, and only if, for any two cells x and y of the hierarchy up to and including the nth level

$$\mathrm{Tl}\text{`}x = \mathrm{Tl}\text{`}y$$
$$\mathrm{Vr}\text{`}x = \mathrm{Vr}\text{`}y$$

and

$$\mathrm{Vol}\text{`}\mathrm{B}\text{`}x = \mathrm{Vol}\text{`}\mathrm{B}\text{`}y$$

If the time-length thus common to all the cells is T, and the common volume-ratio is α, then these two parameters characterize the hierarchy up to and including the nth level. The following table shows the sum of the volumes of the members of the set of 2^p cells for a given level p ($0 \leqq p \leqq n$) of a T,α,n-regular cell hierarchy.

Level p =	Sum of Vols. of initial states of members of pth lev.	Sum of Vols. of final states of members of pth lev.	Time elasping, for any cell x of pth lev., from B‘ zygote to:	
			B‘x	E‘x
0	V_o	$2\alpha V_o$	0	T
1	$2\alpha V_o$	$(2\alpha)^2 V_o$	T	2T
2	$(2\alpha)^2 V_o$	$(2\alpha)^3 V_o$	2T	3T
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
n	$(2\alpha)^n V_o$	$(2\alpha)^{n+1} V_o$	nT	$(n+1)T$

In the above table V_o is the volume of the initial state of the zygote. For any cell x of the nth level $\mathrm{Vol}\text{`}\mathrm{B}\text{`}x = 2\alpha^n V_o$ and $\mathrm{Vol}\text{`}\mathrm{E}\text{`}x = 2\alpha^{n+1} V_o$.

Thus if ξ is the total volume at time t measured from the initial state of the zygote then

$$\xi = V_o e^{\lambda t} \qquad (3)$$

where $t = pT$ and $\lambda = (\log 2\alpha)/T$. For values of t other than integral multiples of T we will assume that the volume is still to be represented by this exponential relation.

Now let us suppose that differentiation with respect to the time-length and volume-ratio of its cells occurs in the $(n+1)$th level of a T,α,n-regular cell-hierarchy. That is to say we assume that N_1 cells of the $(n+1)$th level become beginners of N_1 sub-hierarchies each of which is T_1,α_1,n-regular, where $T_1 \neq T$ and $\alpha_1 \neq \alpha$, while the remaining N_2 cells of the $(n+1)$th level ($N_1 + N_2 = 2^{n+1}$) become beginners of sub-hierarchies which continue to be characterized by T and α. Then at time t from the beginning of differentiation the total volume attained by the cells in the two sets of sub-hierarchies is

$$\xi = N_1 V_o' e^{\lambda_1 t} \tag{4}$$

and

$$\eta = N_2 V_o' e^{\lambda t} \tag{5}$$

where $\lambda_1 = (\log 2\alpha_1)/T_1$, $\lambda = (\log 2\alpha)/T$ and where, if x is any member of the $(n + 1)$th level $V_o' = \text{Vol}^{\cdot}B^{\cdot}x$.

Whence

$$\eta = A\xi^{\kappa} \tag{6}$$

where

$$\kappa = \lambda/\lambda_1 = (T_1 \log 2\alpha)/(T \log 2\alpha_1) \tag{7}$$

and

$$A = (N_2 V_o')/(N_1 V_o')^{\kappa} \tag{8}$$

Equation (6) is the heterogonic growth equation of Huxley. Teissier (1934) gives the equation in the form $z = Kx^{\alpha}$ and states as an experimental fact, that α is independent of the units in which the measures are made, whereas K is dependent on those units. This is seen to be the case from the equation just derived. We have

$$\alpha_{\text{Teiss}} = \text{our } \lambda/\lambda_1, \text{ and } [\lambda/\lambda_1] = 0$$

and

$$K_{\text{Teiss}} = \text{our } A \text{ and } [A] = [\text{Vol}]^{1-\kappa}$$

Needham (1934) says "the equation (Huxley's) has no true physical meaning" (p. 82) because it does not satisfy the criterion of equivalent dimensions. Derived as above the equation is clearly free from this defect and its constants A and κ are observable quantities, at least in simple cell systems.

In the foregoing treatment it is clear that the volume (or weight) measures may be replaced by linear measures without loss of generality, a corresponding modification being made, of course, in the dimensions of A.

Equation (6) is deducible from equations (4) and (5) on elimination of t *whatever* ξ, η, λ_1 λ, N_1, N_2 and V_o' *may represent*. That is to say, the relative growth relation (6) can be exhibited between any two quantities, whether related biologically or not, provided only that each varies with time according to (4) or (5). Thus we can speak of the 'relative growth' of two cell-hierarchies whose cells are parts of two quite distinct organisms (Waddington, 1933) or, more prosaically, we can find the growth rate of B's bank balance (at compound interest) relative to the growth of the bacterial colony under B's microscope. In fact the relative growth equation, as here derived, is simply a mathematical consequence of the customary hypotheses regarding the nature of cell-growth with respect to volume and therefore holds for any entities for which corresponding hypotheses are valid. With a suitable extension of the hypotheses so as to include the division of chemical material in cell-division, and by the use of parameters to denote chemical

volume-change, similar conclusions can be reached regarding "chemical heterogony" (Needham, 1932 and 1934).

It can be shown quite easily that if, say, T_1, α_1 change to new values T_1', α_1', subsequent to the beginning of differentiation, then there is an abrupt change in the constants A, κ. This is the case which gives an abrupt change in the slope of the plot of log ξ against log η (Huxley, Needham, Teissier).

Finally it is of interest to note that much of the relative growth data are such that the two parts compared are largely homogeneous with respect to the cellular material concerned. That is to say, the description in terms of T and α can give a close approximation to the full description of the material.

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THE EFFECT OF MECHANICAL STRESS ON THE DIFFERENTIATION OF SCLERAL CARTILAGE IN VITRO AND IN THE EMBRYO[1]

By

PAUL WEISS and RODOLFO AMPRINO[2]

Department of Zoology, The University of Chicago

The cartilaginous sclera of the eye of a bird is a relatively simple structure, both histologically and morphologically, yet one of well-defined shape and proportions. It appears comparatively late in development and is readily accessible to experimental interventions. For all these reasons, it seems to offer an excellent object for developmental analysis. Part of such an analysis is presented in this paper.

In the chick embryo scleral cartilage is not discernible as such until the end of the first week of incubation. During earlier stages, the eye is surrounded by loose mesenchyme, whose cells are optically indistinguishable from mesenchyme cells in other parts of the embryo. Although their density increases somewhat towards the bulb, with a tendency to tangential orientation, this same feature is observed in the mesenchyme around many other hollow organs, so that it cannot be considered to be specific for the tunic of the eye or indicative of future scleral development. In fact, only part of this denser mesenchyme will later transform into sclera. Figure 1a shows the condition on the fourth day of incubation.

Towards the seventh day the mesenchyme adjoining the pigmented layer of the retina has become very dense, with all cell axes oriented parallel to the surface of the bulb, and on the seventh day it shows the character of pre-cartilage. The formerly elongate cells round up, assume an oval shape, and an acidophilic ground-substance appears between them (Fig. 1b). Toward the end of the seventh day the ground-substance becomes basophilic, and beginning with the eighth day, the typical characters of mature cartilage can be recognized (Fig. 1c). This cartilage, separated from the tapetum by the vascular lacunes of the early choroid coat, has the shape of a curved plate, from three to four cell layers thick. It is bounded by a thin perichondrium. The thickness of the plate increases slightly as one proceeds from the proximal pole of the bulb toward the equator.

This developmental course presents the following problems for analysis: Can the pre-scleral mesenchyme self-differentiate into cartilage?

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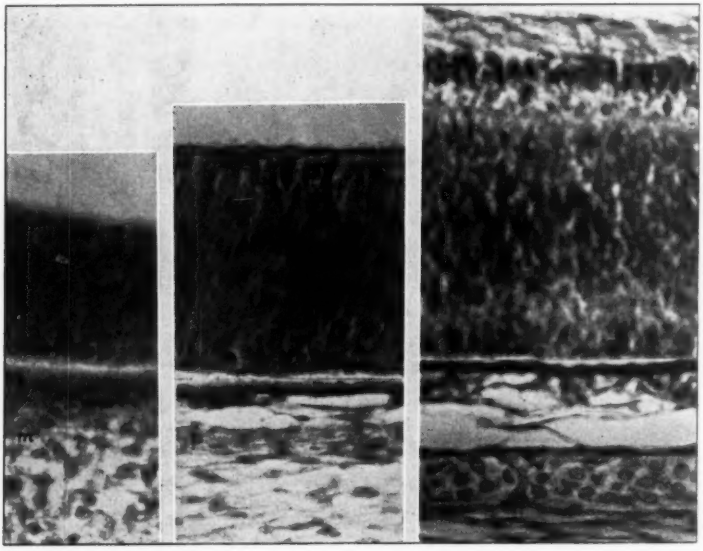
(2) Rockefeller Research Fellow from Torino, Italy.

If so, how early does it possess this faculty? Moreover, does this faculty pertain to morphological as well as to histological characters? Is the eye cup essential for scleral differentiation? If so, in what capacity? Do the tensions produced by the expansive growth of the retina affect the differentiation of the sclera?



a b c

Fig. 1. Three sections through the eye wall of chick embryos of different ages at identical magnifications (× 350).—
a, 4-day embryo. b, 7-day embryo. c, 9-day embryo
Tapetal layers (t) of all three sections lined up. Note transformation of mesenchyme (m, Fig. 1a) into pre-cartilage (p, Fig. 1b) and cartilage (c, Fig. 1c).

Standard methods of tissue culture were used to test self-differentiating capacity and response to mechanical factors. Some supplementary experiments were made on the embryo. The earliest recorded attempts to explant sclera dealt with fully differentiated cartilage (Fischer, 1922). The ability of scleral cartilage to differentiate in vitro, however, was first demonstrated by Weiss and Dorris (1936). Strangeways and Fell (1926) who were the first to study the self-differentiation of the whole chick eye in vitro do not seem to have obtained the formation of cartilage, probably because the eye explants lost most of their mesenchymal coat during successive transfers. The authors simply state that "a certain number of the mesenchyme cells . . . . . gives rise to a loose stroma of connective tissue supporting the epithelial folds

and tubules" of pigmented epithelium. In contrast, eye rudiments cultured without transfer and with the mesenchymal coat left attached (Dorris 1938) produce scleral cartilage quite readily.

In the present experiments the explants consisted mainly of fragments excised from the coat of the eye cup. The adhering pigmented layer was either taken along or peeled off and discarded. The explants were obtained from embryos of various ages from three days of incubation upward. They were imbedded in clots produced by mixing blood plasma and cell-free embryonic juice on cover-slips. Where the effect of mechanical tension was to be studied, tension was applied to the medium during coagulation.

An account of the fate of explanted tapetum in tissue culture is given by Strangeways and Fell (1926) and Dorris (1938). Only the fate of the explanted mesenchyme will be discussed in this paper. Such mesenchyme, when taken from embryos younger than four days of incubation, continues to proliferate in the manner of ordinary connective tissue, without ever giving rise to cartilage. The fourth day is the critical time at which pre-scleral mesenchyme can be shown for the first time to possess definite self-differentiating power.

Fragments explanted on the fourth day or thereafter can produce typical cartilage in vitro, no matter whether tapetum is present or not. This cartilage is not only histologically, but also morphologically of the typical scleral variety. That is, it appears in the shape of a flat plate, bounded by smooth, nearly parallel planes, and covered by a very thin perichondrium (Fig. 12, 13). Time and rate of its appearance in vitro match closely the corresponding values observed in the embryo. The specific staining tests (Mallory-Azan; Thionin) give the reactions typical of normal cartilage.

In short, the undifferentiated mesenchyme surrounding the eye ball of a four-day chick embryo contains all the specific prerequisites for the formation of cartilage of the scleral type, as well as the power to put them into effect in an extraneous medium, independently of the choroid and pigmented layers with which it would normally be associated.

While the qualitative self-differentiating faculty of the fragments at the designated stage is thus well established, variations in the morphology of the end-product do occur, depending on the culturing conditions. The initial size of the explant, as well as the mechanical stresses acting upon it during its differentiation, have a definite effect on the final structure. As for the former, it has been noted that explants below a certain size tend not to differentiate into cartilage. They proliferate actively in the manner of fibroblast cultures, but soon become attenuated and fail to differentiate further. This failure may

be ascribed to the strong horizontal stress to which a culture in a plasma clot is exposed. While a larger piece, owing to the compactness of its fabric, will be able to retain its coherence in the face of disrupting mechanical force, smaller fragments will be more easily pulled apart, thus loosing the tight texture which apparently is a prerequisite of cartilage formation.

This interpretation is further supported by observations on the effect of mechanical stress intentionally applied to cultures. If the intrinsic tensions of a plasma clot are sufficient to disrupt small fragments, the addition of external tensions ought to suppress chondrogenesis even in larger pieces. This is actually the case. The procedure in setting up tensions in the clot is simple and has been used previously in similar studies on the growth of nerves (Weiss, 1934). In the present case, the partly coagulated plasma drop containing the explant is stretched with a needle and held in this position until coagulation has become complete. It then adheres firmly to the cover slip, retaining its deformation, as well as the resulting elastic tensions in its interior.

If cultures of pre-scleral mesenchyme, large enough to produce cartilage under ordinary culturing conditions, are subjected to this treatment, and if the stretch applied is excessive, the explant is drawn out to a very thin layer which merely forms connective tissue without trace of cartilage. The tissue contains heavy bundles of collagenous fibers, oriented in the direction of the lines of stress, which corroborates earlier experiments on mechanical orientation of connective tissue in vitro (Weiss, 1929, Doljansky and Roulet, 1934). However, stains revealed the presence of no chondromucoid, nor did any other characters of cartilage appear. One must conclude, therefore, that excessive stress forces precartilaginous cell masses into a condition in which they can no longer produce cartilage.

The situation is materially different when the stretch applied does not exceed moderate degrees. In such cases, scleral cartilage develops, as it would in unstretched clots. Only the dimensions of this cartilage are now different in that it is thinner than usual. Generally speaking, the thickness of the scleral plate varies inversely with the amount of tension prevailing during its formation. The thickness of the plate is an expression of both the number of cell layers composing it and the amount of ground-substance laid down by them. Evidently, horizontal tension reduces both, as can be readily seen by comparing two areas of the same culture developed under unequal stress (Fig. 12 and 13). The central portion of a stretched culture lying on the surface of the clot is under greater tension than the peripheral zone which is anchored in the clot. Accordingly, cartilage differentiating in the central region (Fig. 13) is much thinner and denser than cartilage developing nearer

the rim (Fig. 12). It is obvious, therefore, that tension acting in the plane of the future cartilaginous plate affects the distribution of the chondroblastic cells, and hence, the thickness of the plate. These observations have suggested some further experiments on the embryo, which will be reported below.

All these results point toward the cells of the pre-scleral mesenchyme as being definitely earmarked for a specific cartilaginous course of differentiation by the fourth day of incubation. Explanted under favorable conditions, they continue in the initiated course. In view of this rigidity, an examination of the regenerative capacities of this cartilage is of some interest. When a cartilaginous plate cut from young sclera is folded and explanted so that the folds come into intimate surface contact with each other, fusion between the parts thus apposed fails to occur, even if there is no perichondrium interposed between them. The individual surfaces remain clearly outlined. It seems impossible, therefore, to abolish the plate-like character of this species of cartilage in favor of a massive bulky kind by the mere stacking up of multiple layers. The lack of regenerative ability indicated by this failure of apposed surfaces to merge is further confirmed by the lack of reparative changes following injury.

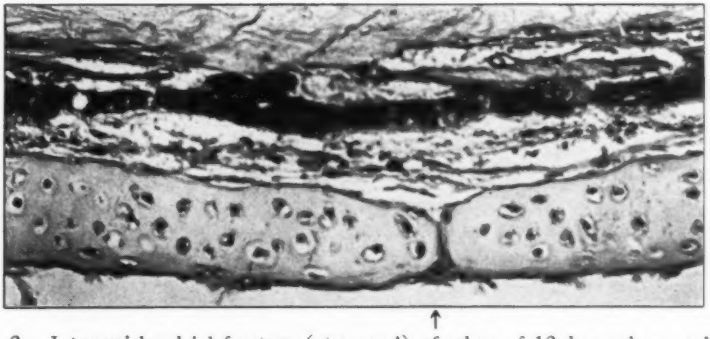


Fig. 2. Intraperichondrial fracture (at arrow!) of sclera of 12-day embryo, cultivated with tapetum for 7 days. No repair.—Mallory-Azan.

When small wedges are cut out from the explanted cartilage, fibroblasts from the undifferentiated part of the explant soon fill the gap. The cartilage cells near the edges of the cut change somewhat in shape, and the ground-substance becomes acidophilic, presumably from loss of chondromucoid. However, no regeneration of cartilage occurs, and the gap persists (Fig. 2). In order to preclude the ingrowth into the gap of fibrous tissue, which might have served as a block to regeneration, the cartilage in some cases was broken intraperichondrially, leaving the perichondrial coat intact. Even so, the fracture failed to heal.

The results are the same, no matter whether one fractures scleral cartilage removed as such from embryos of ten days of age upward, or cartilage differentiated in vitro from younger explants. It seems that the chondrogenic power of the tissue has exhausted itself in the original act of cartilage production.

The internal tensions set up in folded young cartilage have a remarkable effect on its cellular architecture. The influence of mechanical stress on the structure of cartilage is well recognized (Benninghoff, 1931), and a demonstration of this action in vitro was given by Weiss and Dorris (1936). A few supplementary cases were obtained in the present experiments. The results are summarized in the diagram, Figure 3.

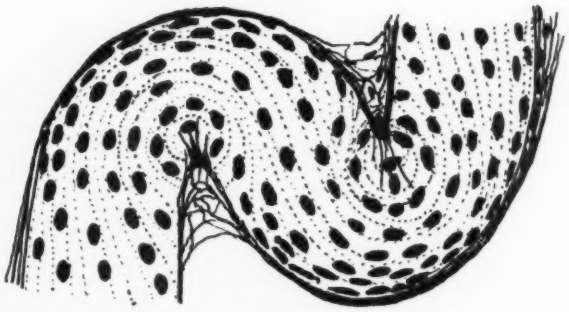


Fig. 3. Diagram illustrating cell arrangement in folded cartilaginous plate. Dotted lines indicate pattern of maximum tensions.

At the convex surfaces, which are under tangential strain, the perichondrial layer is dense, with cells and collagenous fibers running parallel to the surface. On the concave side, the perichondrium loosens up into a stroma from which collagenous fibers radiate into the interior of the cartilage, corresponding to lines of tension produced by the lateral compression of the plate at this side. Inside the cartilage, the distribution of the cells, as well as the orientation of their nuclear axes, reflect very distinctly the pattern of lines of stress, which can be determined by the use of properly shaped rubber plates subjected to the same kind of distortion.

Presumably, the internal tensions act, first, by orienting the precartilaginous fibrous matrix, which then affects the position, orientation and density of the cells in different regions, which, in turn, leads to a differential rate of ground-substance formation. A further analysis of this subject seems desirable and lies well within the scope of tissue culture methods.

The marked thinning of the scleral cartilage in vitro under horizontal stress (see above; Fig. 13) suggested that the development of a sclera of normal proportions in the embryo may hinge upon the presence of the concentric tensions set up by the growth of the eye ball. Ostensibly aided by the increase of the vitreous body in its core, the eye grows rapidly in volume. A crude calculation from sample measurements reveals that between the fourth and the ninth day the volume increases roughly five hundred times. This enormous inflation subjects the pre-scleral coat to stresses similar to the ones applied in our tissue culture experiments. This being the case, it could be expected that by reducing the growth tensions of the bulb in the embryo, one would obtain scleral cartilage of abnormal thickness.

In order to test this assumption, eyes of four-day embryos were pricked and made to collapse. A deep incision was made through cornea and eye wall so that the vitreous humor could flow out. After the operation the shell was closed again, and the embryos were allowed to develop for another two to fourteen days, after which they were fixed and sectioned.

A collapsed retina continues to grow, as does explanted retina, in form of irregular folds. In a normal eye the retina is apparently kept stretched by the turgidity of the vitreous body. Deprived of its support through the draining of the vitreous body, the retina wrinkles up and retains this configuration throughout development. As a result, pricked eyes turn out to be permanently undersized. Figures 4 to 7 show the external features of four heads representing several grades of eye reduction. One notes a striking asymmetry of the skull, with the upper beak deviating toward the reduced side. This fact presents experimental confirmation of the observation that unilateral microphthalmia in chickens is commonly associated with cross-beak (Landauer, 1938).

All constituents of the collapsed eyes are affected to some degree, but it is beyond the scope of this paper to deal with them in detail. In passing, it may be mentioned that, while the optic layer of the retina folds up, the pigmented layer remains straight. It thus reveals itself as being a membrane of great elasticity and low active growth tendency, which is enormously stretched out in a normal eye, but proportionately contracted in a collapsed one.

In a sense, the fate of the scleral mesenchyme is very similar. The cartilage around the operated eye is essentially co-extensive with whatever surface the reduced bulb has assumed. Its thickness, on the other hand, is much greater than that of the normal sclera of the opposite, unoperated, eye. It may be as much as six times thicker. Figure 8 illustrates the general situation, and Figures 9 to 11 show three cases

under higher magnification. The thickness of the cartilage around reduced eyes, while varying locally, presumably according to local variations in the growth pressure of the retina, never seems to attain the thinness of normal sclera.

The change in scleral proportions in the collapsed eyes is precisely what was to be expected from the tissue culture experiments: A certain mass of circumbulbar mesenchyme of the four-day embryo is earmarked for scleral differentiation. While in normal development the growing eye stretches this mass to increasingly greater surface expansion at the expense of depth, the loss of distension in the operated eyes has arrested this thinning process. The same mass of cartilage has been cast, as it were, into a larger and thinner plate in the former case, and into a smaller and thicker plate in the latter. It is well to keep in mind, however, that only the general inverse relationship between surface expansion and thickness has actually been observed, while no accurate measurements and volume computations have been attempted.

The mode in which the scleral cartilage increases during the stages following the ones discussed in this paper has not been studied, and it cannot be predicted what corrective effects, if any, later growth might have on the proportions of the deformed sclerae.

DISCUSSION

Generally speaking, the formative potencies of any given embryonic area are the wider, the earlier the stage at which it is tested. Development implies the progressive fixation of divergent trends in different regions of the body, attended by gradual restriction of formative

Figs. 4-7. Heads of embryos with experimental microphthalmia on right side. A comparison of the darkly pigmented eyes (Fig. 4 and 5) shows the great difference in size between the normal and collapsed bulbs.

Fig. 8. Frontal section through the head of a 12-day embryo whose right eye (left in the picture) had been punctured on the 4th day.—× 14.—Thionin.—
S, Sclera T, Tapetum nigrum R, Retina M, Median septum.

Figs. 9-11. Frontal sections of interorbital region of embryos whose right eyes (left in the pictures) had been punctured on the 4th day. Diameters of scleral cartilages marked by black lines on upper margin of each figure. Median septa marked by dotted lines.

Fig. 9. Embryo of 12 days.—× 62.—Thionin.

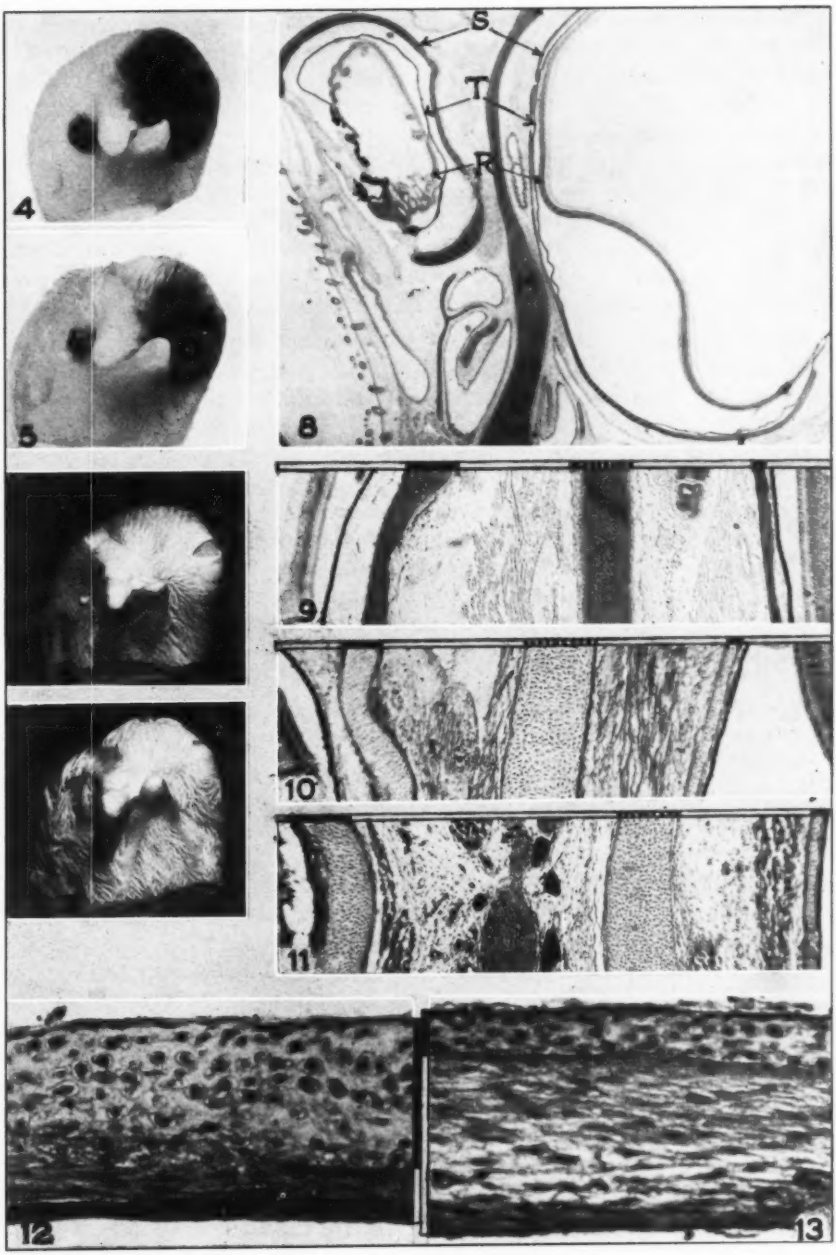
Fig. 10. Embryo of 10 days.—× 78.—Mallory-Azan.

Fig. 11. Embryo of 13½ days.—× 65.—Mallory-Azan.

Figs. 12 and 13. Cross sections through two regions of the same scleral culture under different degrees of stress. Explant taken from 7-day embryo and cultivated for 4 days in stretched clot.—× 350.—Mallory-Azan.

Fig. 12. Peripheral portion of culture, where tension reaches a minimum.

Fig. 13. Central portion of culture, where tension reaches a maximum.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capacities. This concept of development has by now become firmly established (cf. Weiss, 1939). The changes occurring in an embryonic field which lead to this gradual fixation and which are commonly referred to as "determination", antedate the appearance of manifest differentiation. The microscopically discernible criteria by which we commonly tell differentiations appear as late expressions of preceding differentiating processes, for the detection and identification of which we have as yet found no appropriate methods. What has happened in differentiation can be judged only by the outcome, and by the time the outcome becomes perceptible, a major part of the differentiating processes have become a matter of the past.

Applied to explants, this means that the only way to determine what kind of differentiating activities may have been under way in a given fragment at the time of isolation, is to wait and see into what structure it is going to develop. If two fragments which to all appearances are alike, nevertheless, under otherwise identical conditions, give rise to different structures, they could not possibly have been identical, to begin with, all appearances to the contrary notwithstanding. They must have differed in some regards—e. g., as for substances, structures, forces, rates of reactions, etc.,—even though the differences may not have been detectable by the methods of optical inspection and microchemical tests now at our disposal. It is to be expected that the conventional distinction between "determination", i. e., implicit differentiation (see also Bloom, 1936), and "manifest differentiation" will fade, as our physical and chemical methods of discrimination become more sensitive and refined. Methods revealing orderly molecular arrangements, particularly in surface films, may assume increasing importance in this field.

"Determined", while not "manifestly differentiated", is precisely the condition in which the pre-scleral mesenchyme appears on the fourth day of incubation. At that time, it is histologically indistinguishable from mesenchyme from other embryonic sources; however, when explanted, it proves to contain all the specific factors accounting for its turning into sclera, rather than into other mesenchymal derivatives. Mesenchyme of the three-day chick, on the other hand, has given negative results. Such younger tissue has obviously either not yet received the specific scleral ear-mark, or if it has, lacks the power to put the differentiation through under the adverse conditions of tissue culture.

How the circumbulbar mesenchyme receives its determination for the scleral course, cannot be said. It may owe it to an autonomous process (cf. Weiss, 1939, p 314) or, more likely perhaps, to an inductive action by the enclosed young eye cup. The latter alternative is sug-

gested by some results of Twitty (1932) in amphibians: Transplanting embryonic eye cups between a fast-growing large-eyed and a slow-growing small-eyed species of Amblystoma, he observed a tendency of the sclerae produced by host mesenchyme to match the size of the bulbs. If the situation in the chick and in amphibians is essentially the same, Twitty's observation would indicate that eyes of higher growth potential cause a greater amount of mesenchyme to be deposited and earmarked for scleral development than eyes of slower growth. We do not know, however, whether the mode of growth of the scleral cartilage is really the same in amphibians and birds.

Whether the "determined" condition which characterizes the pre-scleral mesenchyme of the four-day chick embryo is merely a property of the explanted fragment as a whole, or whether it applies to each individual cell, has not been decided. It is quite possible that the cells at the time of their explantation are still fairly labile, but that some "field" condition present in their fabric, such as, for instance, a definite surface orientation, or some "pacemaker" condition, may be carried over into the culture with the piece as a whole, and there later puts its impress on the constituent cells, according to the principle of "progressive determination" (see P. Weiss, 1939, p. 321). To test this point, one would have to liberate small cell groups from their mutual interconnections, transplant them to a variety of other fields, and examine their ability or inability to differentiate in conformity with the new conditions. The fact that a mass of pre-scleral mesenchyme invariably produces cartilage, does not yet prove that the individual cells of the same stage would also have done so when reared independently.

By the seventh day the cells have evidently become definitely differentiated, as is indicated by the lack of regenerative ability at this and somewhat later stages. Wounds afflicted to the young cartilage fail to heal. Neither is there production of new cartilage from the cut surfaces, nor does the fibrous tissue moving into the wounds become chondrified. A recruitment of fibrocytes by cartilage explanted in vitro, with ensuing transformation of some of the fibrous fringe into cartilage, has been reported (A. Fischer, 1932), but nothing of the sort was seen in our present objects. The absence of regeneration in our cases is in general agreement with the observations of Niven (1931) on the healing of fractures in explanted embryonic limb cartilage: Although very young limb cartilage, when fractured before explantation, did proliferate across the gap, cuts made some time after explantation were repaired by fibrous and osteoid tissue without active participation of the severed cartilage.

It must be stressed that the self-differentiating pre-scleral mesen-

chyme in our experiments has produced not merely cartilage in a general histological sense, but has actually elaborated the typical morphological characteristics of sclera. Patella developed in vitro has likewise been observed to assume a semblance of normal shape (Niven, 1933). This is noteworthy because usually the morphogenetic development of explants—and the same holds true for transplants to the chorio-allantoic membrane—is much poorer than their histological differentiation. Deficiencies of shape occurring under these conditions, however, can be easily accounted for by the peculiar spatial and mechanical conditions of the new environment which present physical obstructions to the proper shaping of the explants; whereas, in the case of the sclera, the physical conditions of the culture medium are evidently compatible with the elaboration of the typical morphological pattern.

The similarity between the tensions around a growing eye and those prevailing in a plasma clot has been discussed in the text. All evidence, gained both from the imposition of added tensions upon the clot, and from the release of the normal growth tensions in the eye of the embryo, points toward the participation of stress in the molding of the prescleral mesenchyme and scleral cartilage. But it must be kept in mind that these tension effects merely modify the dimensions and internal structure of a tissue which is per se disposed to form cartilage, while they are in no way the primary factors calling forth cartilaginous development as such. As a matter of fact, as has been shown, strong tensions preclude rather than enhance chondrogenesis.

Whether tensions of moderate degrees acting on the sclera affect only the spatial disposition of the cells or also their growth rates and secretion rates, and, hence, the total volume produced, cannot be decided, as precise measurements are lacking. With regard to other tissues, however, the indications are that tension acting during the growth phase may have a decided influence on growth rate. This fact is illustrated by the retinae of collapsed eyes (Fig. 8). Such retinae have remained definitely undersized. Evidently, retina grows faster when under distension than when loose. This might be explained as follows: Proliferation in a young retina is essentially confined to the cells along the outer surface (facing the tapetum nigrum). Part of the newly proliferated cells remain in this surface, swelling the source of further proliferation, while the rest move into the deeper layers, where they differentiate and cease to reproduce. Expansion of the retina by stretch permits more cells to occupy superficial positions than could find room in the unstretched surface. Thus, stretch, by expanding the source of cellular proliferation, raises the rate of increase of the retina. This we may assume to be the reason why a collapsed retina,

deprived of the usual distension effect of the swelling vitreous body, grows at a reduced rate. It is not unlikely that similar influences of distension on growth rate could be demonstrated in many other organs.

SUMMARY

(1) The cartilaginous sclera of the chick assumes its differentiated aspect by the seventh day of incubation. Prior to that time, the eye is surrounded by loose mesenchyme of no recognizable distinction.

(2) When fragments of this mesenchyme are explanted in vitro prior to the fourth day of incubation, they continue to grow in the manner of ordinary fibrocytes.

(3) When explanted on or after the fourth day, they produce typical scleral cartilage. The rate of differentiation in vitro corresponds closely to that in the embryo.

(4) Explanted cartilage of this variety is devoid of regenerative capacity.

(5) Strong tensions tend to suppress the differentiation of chondrogenic tissue into cartilage.

(6) Moderate tensions affect the dimensions of the differentiating cartilaginous plate; the stronger the tensions, the thinner the plate.

(7) The internal cell and fiber architecture of bent cartilage reveals molding effects of mechanical stresses.

(8) Embryonic eyes caused to collapse by pricking on the fourth day develop sclerae of excessive thickness, owing to the absence of the tensional thinning effect normally exerted by the expansive growth of the bulb.

(9) Embryos with unilateral microphthalmia develop cross-beak.

(10) Retinae of collapsed eyes are undersized, evidently owing to lack of distension. This indicates a definite relation between stretch, size of proliferating surface, and growth rate.

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THE PRENATAL GROWTH OF THE CAT
X. THE WEIGHT OF THE SPLEEN IN THE FETAL PERIOD AND IN THE ADULT

By

HOMER B. LATIMER

Department of Anatomy, University of Kansas

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The weights of the human spleen have received a good deal of attention in recent years and the one uniform feature in most of these reports is the variability in the weight of the spleen. The studies on its function have explained these variations to a degree and yet its variability seems to be more or less of a challenge for the establishment of a 'normal weight'. No attempt will be made to review all of this literature. Bean and Baker (1919), Bean (1926), Ahronheim (1937), and Krumbhaar and Lippincott (1939) have made extensive studies of the human spleen and have reviewed the earlier literature. Hwang, Lippincott and Krumbhaar (1938) have studied the quantitative postnatal changes in the weight of the spleenic lymphatic tissue in man of different ages. Barcroft et al (1925) have studied the weights of the spleen in adult cats killed by different methods. There is still need for further quantitative studies of the spleen in various animals and with this in view this little report on the spleen in the cat is presented.

MATERIALS AND METHODS

The spleen was not weighed in 15 of the smaller fetal cats, although the spleens of some of the smallest fetuses were weighed and thus we are able to get the formulae for the entire range of body weight and body length. There were 214 fetal spleens, 35 newborn spleens and the spleens from 104 adult cats. The methods used are the same as in the preceding numbers of this series. The spleens were carefully dissected out and all mesenteries and vessels cut close to the organ. They were weighed in glass stoppered weighing bottles on a chemical balance sensitive to 1/10 milligram. The empirical formulae were developed as in the preceding papers of this series and the formulae used in getting the statistical values are those given by Dunn (1929).

SPLEEN WEIGHTS

The weights of the fetal and the newborn spleens, plotted on body weight and on body length are shown in Figure 1. The curve starting farther to the left and with the individual spleens shown as circles and the newborn spleens as double circles was drawn from the following formulae, from 0.6 to 90 grams of body weight,

$Y = \frac{(0.1X)^{1.4}}{105.7}$, and from 90 to 200 grams of body weight,

$Y = 0.00114X + 0.1024.$

In the above formulae, Y represents the spleen weight in grams and X, the body weight in grams. The average percentage deviation of the calculated from the observed averages for each ten grams increase in body weight for these two formulae is 9.01 per cent. This is a higher percentage than has been reported for many curves and yet when the scatter of the individual cases, as shown in this figure, is taken into consideration this is not a high average percentage deviation.

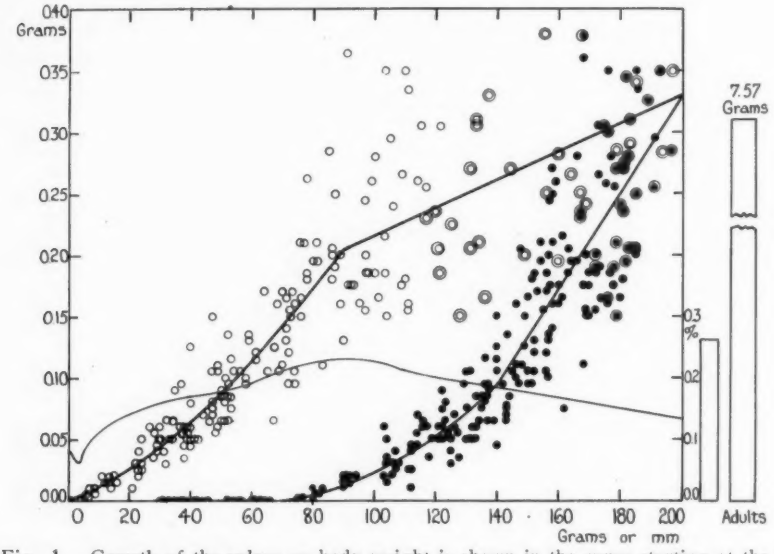

Fig. 1. Growth of the spleen on body weight is shown in the curve starting at the left and with the cases for the fetal spleens shown as circles and the newborn spleens as double circles. The growth of the spleen plotted on body length is shown by the curve starting farther to the right and with the individual fetal spleens shown as dots and the spleens of the newborn cats shown as dots enclosed by circles. Empirical formulae from which both curves were drawn are given in the text. The taller broken column represents the average weight of the adult male spleen. The lighter line without any cases represents the percentage weight of the spleen plotted on body weight. The shorter column at the right represents the percentage weight of the adult male spleen. The percentage values are given at the right and the weights of the spleens in grams at the left.

The second curve, or that starting farther to the right and with the individual spleen weights shown as dots for the fetal cats and as dots enclosed by circles for the newborn cats, from 70 to 140 millimeters of body length was drawn from the formula,

$Y = \frac{(0.1X)^{2.7}}{11190} - 0.0176$, and from 140 to 200 mm. of body length,

$Y = 0.004X - 0.4665$.

In these formulae, Y again represents the spleen weight in grams and X, now represents the body length in millimeters. The average percentage deviation of the calculated from the observed averages for each ten millimeters increase in body length is 5.50 per cent. As there was a great deal of variation in the weights of the spleens below 70 millimeters of body length, the above formula does not fit these very well and so 70 millimeters is given as the lower limit for the formula.

It is interesting to see that for both body length and for body weight the initial parts of the curves are concave superiorly and each terminates as a straight line curve. The spleen grows at an increasingly rapid rate at first compared to the growth of body weight and, with a more rapid acceleration when plotted on body length.

From thirty to seventy millimeters of body length there is a very slow increase in the weight of the spleen and it was this part which could not be well represented by the formulae. The increase in spleen weight follows the increase in body weight more closely than the body length. Over half of the total increase in spleen weight comes during the last third of the increase in body length.

The spleens of the 52 males and 52 females were studied statistically with the following results:

	Males	Females
Average weight	7.57 ± 0.37	5.67 ± 0.23
Coef. of variation	51.56 ± 4.22	43.17 ± 3.35

The significant ratio, or the difference between the average weights of the males and the females divided by its probable error was found to be 4.40, which would indicate statistically a significantly larger spleen in the male cat. The coefficient of variation of 51.56 per cent in the males is the largest coefficient found for the organs of the cat thus far, with the exception of the coefficient of 55.68 per cent for the testes (Latimer, 1939a). The male organs of the cat have, in general, been found more variable as are the male spleens.

Barcroft et al (1925) give the weights of the spleen of several cats after different methods of killing. The weights differ but in general they are much heavier than the spleens in my cats.

To give a better method of estimating the adult spleen weight from body weight or body length, empirical formulae have been prepared as has been done for some of the other papers of this series and the formulae for the determination of the spleen weight from either body

weight or body length are given below. For the males, from 1700 to 4500 grams of body weight,

$Y = 0.00307X - 1.232$, and for the females from 1500 to 3500 grams

$Y = 0.0031X - 1.9211$.

In both of these formulae, Y represents the weight of the spleen in grams and X, the body weight also in grams. The average percentage deviation of these formulae are 8.23 per cent for the males and 2.37 per cent for the females. Both of these are straight line curves, as are the two following and so all of the curves for the adult cats will be omitted.

The same spleen weights plotted on body length gave straight line curves which were drawn from the following empirical formulae:

$Y = 0.053X - 20.197$, for the males from 450 to 560 mm. and,

$Y = 0.04X - 14.586$, for the females from 450 to 560 mm. of body length. In both of these formulae, Y represents the spleen weight and X, the body length in millimeters. The average percentage deviation of the calculated from the observed averages is 5.89 per cent for the males and 5.52 per cent for the females. There was more scatter in the males than in the females when plotted on body weight and on body length, and the male spleen is significantly heavier and is also more variable than the female spleen.

PERCENTAGE WEIGHTS

The percentages of the spleen weights were determined for each of the fetal, the newborn and the adult cats. The percentage weights of the spleens up to and including the newborn were plotted on body weight and a curve drawn through the averages for each ten grams increase in body weight. This curve without the individual cases is represented as the lighter line in Figure 1. The maximum percentages seem to come at about 90 grams of body weight with a decrease in the late fetuses and newborn cats. This could be explained in the newborn as the result of trauma. From the smallest fetuses weighing 0.3 gram up to about 4 grams of body weight there is a perceptible decrease in the percentage weight of the spleen, and from four grams on there is an increase, more rapid at first and then gradually increasing to the maximum at 90 grams of body weight.

The percentages of the adult spleens have been studied statistically, and they are as follows:

	Male	Female
Av. percentage weight	0.264 ± 0.010	0.230 ± 0.007
Coefficient of variation	41.14 ± 3.15	33.76 ± 2.47

The significant ratio for these percentage weights is 2.77 which means that there is possibly a significantly greater male spleen weight in terms of percentage of the body weight. The percentage weights are less variable than the coefficients of the absolute weights but they are still greater than the coefficients of the body weights which are 25.01 and 21.07 for the males and females respectively (Latimer, 1936).

The percentage weights of the adult spleens were plotted against body weight as were the absolute weights. To save space the curves are not shown. For the males the curve starts at about 0.24 per cent and after a slight decrease rises to its maximum of 0.34 per cent in the cats of about 3300 grams of body weight and then slowly decreases to about its starting level in the cats of 4500 grams of body weight. For the females, the curve is a straight line rising from 0.19 per cent at 1500 grams of body weight to its maximum of 0.25 per cent at about 2800 grams of body weight and then it decreases slightly to 0.24 per cent at 3500 grams of body weight. It is very likely that these largest cats were older cats and if this is true, this terminal decrease in both sexes would agree with the findings of Hwang et al (1938) for the older adult human spleen. They show the highest peak between birth and ten years but unfortunately we know of no data on the cat spleen between birth and maturity.

These percentage weights of the spleen would indicate a maximum relative weight towards the end of the fetal period with the average adult spleen still heavier. In both the males and females, the spleen is relatively heavier in the cats slightly above average body weight.

TABLE I
CORRELATIONS WITH SPLEEN WEIGHT

	Males	Females
Body weight	+ 0.646 ± 0.055	+ 0.616 ± 0.059
Body length	+ 0.470 ± 0.073	+ 0.371 ± 0.081
Hypophysis	+ 0.081 ± 0.094	+ 0.110 ± 0.083
Thyroid	+ 0.405 ± 0.078	+ 0.461 ± 0.074
Suprarenals	+ 0.491 ± 0.072	+ 0.289 ± 0.086
Gonads	+ 0.572 ± 0.063	+ 0.443 ± 0.076
Uterus	— — —	+ 0.392 ± 0.083
Liver	+ 0.367 ± 0.081	+ 0.194 ± 0.090
Pancreas	+ 0.367 ± 0.081	+ 0.362 ± 0.081

CORRELATIONS

The correlations between the spleen weight and the weights of some of the endocrine glands, the liver and the pancreas are shown in Table I. The average of the male correlations is +0.425 and the females average +0.360 or 18 per cent greater in the males. As has been found in most of the organs of these adult cats, the males are more variable

and yet the correlations are higher in the males. The highest correlations are between the spleen weight and the body weight in both sexes and in both sexes the lowest correlations are with the weight of the hypophysis.

Perla (1936) finds that the removal of the hypophysis in the rat causes progressive atrophy of the spleen and Twort and Lyth (1940) state that there is a positive correlation between the hypophysis and the spleen in mice. They did not employ the usual statistical methods and so their conclusion is somewhat vague. The correlation between the weight of the hypophysis and the spleen of the cat is positive but so low that it is not significant, just as has been reported for the correlations of the hypophysis and the weights of the endocrine glands (Latimer, 1939a). It would seem that the spleen which is primarily an organ of the vascular system should have a better correlation with the weight of the liver. This correlation with the liver in the female cats is especially low and its probable error is nearly one-half the correlation. The only correlations with the weight of the spleen, given in Table I, above +0.5 per cent are with the body weight in both sexes and with the weight of the testes.

ACCESSORY SPLEENS

Accessory spleens have been reported frequently, so this will merely add more cases to the already rather long list. In the fetal and newborn cats two accessory spleens were found, both in females. One fetus weighed 32.98 and the other 97.53 grams. In both of these cases the total spleenic weight was well within the limits of variability but slightly below the average. It is possible that other small accessory spleens were missed in the dissections of the smaller fetuses. There were four accessory spleens found in the 104 adult cats. One of the females had a total spleen weight very slightly above the average and the other female a little below the average. The two males were likewise, one above the average and the other below but both within the range of variability so that in general, in all of these cases of accessory spleens the total spleenic tissue was about the average amount. The accessory spleens were located either closely adjacent to the spleen, or at some distance. The one most remote from the spleen was in one of the adult males and located in the greater omentum near the ventral abdominal wall.

SUMMARY

Empirical formulae for the determination of the spleen weight from either body weight or body length are given for the fetal period and for the newborn cats. Separate formulae are given for the males and the females in the adult cats.

The male spleen is significantly heavier in absolute weight and possibly heavier in percentage weight. Both sexes have very variable spleens, with the males the most variable, in both absolute and in percentage weights.

The spleen weight is well correlated with the body weight in both sexes and with the weight of the testes but the correlations with the other endocrine glands and with the liver and the pancreas are all positive but not significant. The lowest correlations, positive but not significant, are with the hypophysis in both sexes.

Accessory spleens were found in two fetal cats, both females and in two adult males and two adult females.

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EMPIRICAL FORMULAE FOR THE MEASUREMENTS OF THE CENTRAL NERVOUS SYSTEM AND OF THE DIGESTIVE SYSTEM IN THE ADULT CAT

By

HOMER B. LATIMER

Department of Anatomy, University of Kansas

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The use of the average or the mean together with its error has been and still is, probably the best method of expressing the central tendency of a random collection. In the study of any organ taken from a random collection of any species of animal, the average and its error give probably the best idea of this measurement in an idealized or average animal. This average measurement taken from an animal obviously above or below the average body weight or body length would naturally be either too high or too low for the particular animal studied and hence it has seemed that a simple formula based on body weight or body length would better predict the weight or linear dimension of the organ for all animals except the animal which happened to be an 'average animal' in weight and body length.

The average weights and linear dimensions of the digestive system and of its divisions (Latimer, 1937) and of the central nervous system and of its parts (Latimer, 1938) have been published, but in order to provide a more accurate method of predicting 'normal' dimensions in adult cats of various sizes these formulae are presented.

MATERIALS AND METHODS

The data used in this study are the same as those used in preparing the first table in each of the two papers referred to above, and include the weights and linear dimensions of the digestive system and the central nervous system of 104 adult cats equally divided as to sex. The weights and linear dimensions were plotted on body weight and also on body length and then the average weight or length of the organ was found for each 500 grams of body weight or 25 millimeters increase in body length. These averages for each unit of body weight or body length were then plotted, together with the individual data on the field graphs and curves were drawn as closely as possible through these averages. Next an empirical formula was derived for each curve. Later on the differences between the calculated and the observed values will be discussed and in all cases the observed values are these averages.

THE CENTRAL NERVOUS SYSTEM

The brain, the cerebellum and possibly the prosencephalon show significant sex differences in their average weights as shown in the earlier paper, but the body weight and possibly the body length (nose-anus length) are greater in the male cat (Latimer, 1936) and so all of these organs have been studied separately in the two sexes. All of the curves for both the central nervous system and also for the digestive system are straight line curves except the curves in the female cats for the weights of the brain, the spinal cord and the prosencephalon. To save space and also because it is hardly necessary, the curves will not be shown.

The formulae for the weights of the parts of the nervous system plotted on body weight will be given first. In all of the formulae Y represents the weight of the organ or division of the brain in grams and X represents the body weight also in grams. The body weights of the male cats ranged from 1700 to 4500 grams and these formulae have been determined for this range of body weight only. For the females the range of body weight and the limits of the formulae are from 1500 to 3500 grams of body weight.

The following are the formulae for the males:

Brain,	$Y = 0.00136X + 23.688,$
Cord,	$Y = 0.00121X + 4.594,$
Eyeballs,	$Y = 0.00093X + 7.155,$
Prosencephalon,	$Y = 0.00093X + 17.919,$
Mesencephalon,	$Y = 0.000135X + 0.773,$
Cerebellum,	$Y = 0.00016X + 3.478,$
Medulla,	$Y = 0.0002X + 1.309.$

The average percentage deviations of the calculated from the observed averages for the above formulae are as follows: brain, 1.26; cord, 1.32; eyeballs, 1.20; prosencephalon, 1.04; mesencephalon, 3.71; cerebellum, 2.20 and the medulla, 1.85 per cent.

The following formulae are for the females from 1500 to 3500 grams of body weight:

Brain,	$Y = 8(\log X) - 0.512,$
Cord,	$Y = 3(\log X) + 0.0007X - 4.335,$
Eyeballs,	$Y = 0.00105X + 7.125,$
Prosencephalon,	$Y = 6(\log X) - 0.377,$
Mesencephalon,	$Y = 0.0001X + 0.883,$
Cerebellum,	$Y = 0.00031X + 3.077,$
Medulla,	$Y = 0.00017X + 1.373.$

The average percentage deviations of the calculated from the observed averages for the above formulae are as follows: brain, 1.15; cord, 1.65;

eyeballs, 1.17; prosencephalon, 1.30; mesencephalon, 2.69; cerebellum, 1.38 and the medulla, 1.49 per cent.

The following formulae give the weights of the parts of the nervous system of the males and females based on body length. In these formulae Y, as before, represents the weight of the part and X now represents the body length in millimeters. The formulae for the males from 450 to 610 millimeters are:

Brain,	$Y = 0.006X + 24.24,$
Cord,	$Y = 0.023X - 4.05,$
Eyeballs,	$Y = 0.023X - 2.305,$
Prosencephalon,	$Y = 0.0024X + 19.17,$
Mesencephalon,	$Y = 0.0019X + 0.137,$
Cerebellum,	$Y = 0.0017X + 3.055,$
Medulla,	$Y = 0.0032X + 0.189.$

The average percentage deviations of the calculated from the observed averages are as follows: brain, 2.61; cord, 5.36; eyeballs, 2.48; prosencephalon, 3.23; mesencephalon, 3.24; cerebellum, 1.61 and the medulla, 2.39 per cent.

For the weights of the same parts in the female cat and plotted on body length from 450 to 560 millimeters, the formulae are as follows:

Brain,	$Y = 0.0427X + 4.785,$
Cord,	$Y = 0.033X - 9.15,$
Eyeballs,	$Y = 0.03X - 5.50,$
Prosencephalon,	$Y = 0.018X + 10.8,$
Mesencephalon,	$Y = 0.00218X + 0.02,$
Cerebellum,	$Y = 0.01X - 1.30,$
Medulla,	$Y = 0.0062X - 1.358.$

The average percentage deviations of the calculated values in these formulae from the observed average values are as follows: brain, 0.74; cord, 1.35; eyeballs, 2.11; prosencephalon, 1.44; mesencephalon, 1.58; cerebellum, 1.86 and the medulla, 2.48 per cent.

The averages of the percentage deviations show that the averages for the males on both body weight and on body length are higher than the corresponding groups for the females, or the fit of these formulae is better in the female cats. When we compare the fit on body weight and on body length we find that the males average 66.4 per cent more variable when plotted on body length. The formulae for the females are also less well fitted when plotted on body length but the difference here is but 6.99 per cent. All of these formulae however fit the observed averages very well, since the highest average deviation is but 5.36 per cent and this is the only average deviation above 4 per cent.

There was a good deal of scatter in the individual cases when they were plotted. These formulae should give a closer approximation to the true weight of any of these parts than the average values as given in the previous report.

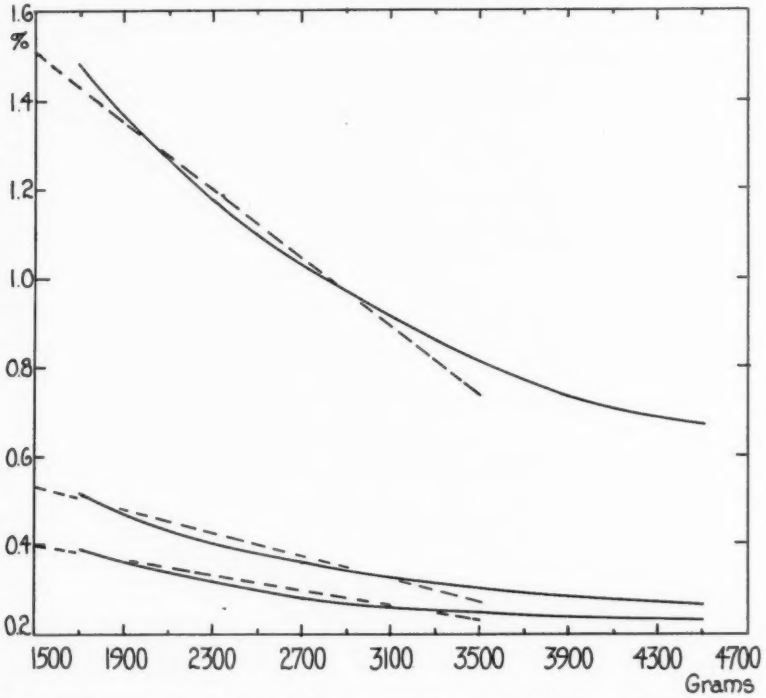

Fig. 1. Percentages of body weight and plotted on body weight. Solid lines represent percentages in the males and broken lines, in the females. Upper pair of curves represent the percentages of the brain weight, middle pair of curves, the percentage weights of the two eyeballs, and the lowest pair, the percentages of the spinal cord.

The weights of the brain, the eyeballs and the spinal cord are given in the earlier paper as percentages of the body weight, but these are average percentages of the entire group and, to see how the percentages of these three organs varied in the adult cats, the individual percentages were plotted and averages for each range of body weight or body length were obtained in the same manner as for the absolute weights. No formulae were developed but the smoothed curves drawn through the averages are shown in Figure 1. No individual percentages are given.

It is interesting to see that for all three organs the percentages of the

males are curves concave superiorly while the percentages of the females are straight lines. All three organs are relatively larger in the smaller cats, but there is more difference in the percentages of the brain, than in the eyeballs or the spinal cord. The correlations between the body weight and the brain weight, as shown in Table II of the earlier report, are much lower than the correlations for the weights of the eyeballs and the cord with body weight. In other words the size of the eyeballs and the spinal cord tend to follow the size of the cat while the weight of the brain is more or less independent of the weight of the entire cat, thus it would form a larger percentage of the body weight in the smaller cats. While getting the body weights and the organ weights of these cats, this apparent independence of the size of the brain and the body weight was noticed and now the statistical evidence confirms these observations. A series of over 300 dog brains is being studied. As yet the statistical evidence is not available but in making the weighings the same lack of similarity between brain weight and body weight has been observed. This variation in brain size independent of the body size would seem to indicate a variation in intelligence in the animal, provided a quantitatively larger brain means a more intelligent brain. Naturally our data give no answer to this suggestion.

THE DIGESTIVE SYSTEM

Formulae for the weights of the digestive tube, of its parts, of the liver and of the pancreas have been prepared in a manner similar to that used for the nervous system. In addition, formulae have been prepared for the lengths of the digestive tube and of three of its divisions. The term 'digestive tube' is used to indicate the digestive system exclusive of the large extrinsic glands, or it includes the esophagus, stomach, small and large intestine. In the first four of the following groups of formulae, Y represents either the weight of the organ in grams or the length in millimeters, and X represents the body weight in grams. As stated above, the range of body weights is from 1700 to 4500 grams for the male cats. The following are the formulae for the weights of the male organs plotted on body weight:

Digestive tube,	$Y = 0.024X + 62.00,$
Esophagus,	$Y = 0.0014X + 2.053,$
Stomach,	$Y = 0.0048X + 9.671,$
Small intestine,	$Y = 0.0137X + 41.99,$
Large intestine,	$Y = 0.0038X + 9.65,$
Liver,	$Y = 0.0215X + 36.85,$
Pancreas,	$Y = 0.0015X + 2.35.$

The average percentage deviations of the calculated from the observed averages for the above formulae are as follows: digestive tube, 2.45:

esophagus, 2.74; stomach, 3.01; small intestine, 3.15; large intestine, 1.86; liver, 4.57 and the pancreas, 3.94 per cent.

The formulae for the weights of the female organs plotted on body weight from 1500 to 3500 grams are as follows:

Digestive tube,	$Y = 0.023X + 64.883$,
Esophagus,	$Y = 0.0015X + 1.65$,
Stomach,	$Y = 0.0038X + 11.80$,
Small intestine,	$Y = 0.0108X + 47.859$,
Large intestine,	$Y = 0.0047X + 7.530$,
Liver,	$Y = 0.022X + 34.021$,
Pancreas,	$Y = 0.00165X + 2.416$.

The average percentage deviations for these are as follows: digestive tube, 0.71; esophagus, 3.47; stomach, 2.83; small intestine, 2.12; large intestine, 0.34; liver, 2.50, and the pancreas, 1.61 per cent.

In the following formulae, the lengths in millimeters of the male organs are plotted on body weight from 1700 to 4500 grams. Y now represents the organ length in millimeters.

Esophagus,	$Y = 0.011X + 161.22$,
Small intestine,	$Y = 0.098X + 1143.4$,
Large intestine,	$Y = 0.0364X + 160.142$,
Digestive tube,	$Y = 0.09X + 1777.0$.

The average percentage deviations for these linear dimensions are: esophagus, 0.87; small intestine, 2.15; large intestine, 3.40 and the digestive tube, 1.64 per cent.

The empirical formulae for the linear dimensions of the females in millimeters plotted on body weight from 1500 to 3500 grams are as follows:

Esophagus,	$Y = 0.0117X + 161.27$,
Small intestine,	$Y = 0.1X + 1100.0$,
Large intestine,	horizontal line average 253.687 mm.,
Digestive tube,	$Y = 0.165X + 1523.93$.

The average percentage deviation for these formulae are: esophagus, 1.30; small intestine, 0.74 and for the digestive tube, 0.74 per cent.

In the following formulae X represents the body length (nose anus length) measured in millimeters, and Y as before either the organ weight in grams or the organ length in millimeters. The weights of the male organs plotted on body length from 450 to 610 millimeters for all of the organs are:

Digestive tube,	$Y = 0.46X - 108.303$,
Esophagus,	$Y = 0.0243X - 6.679$,
Stomach,	$Y = 0.071X - 13.65$,
Small intestine,	$Y = 0.3X - 74.42$,
Large intestine	$Y = 0.064X - 12.322$,
Liver,	$Y = 0.354X - 83.99$,
Pancreas,	$Y = 0.0238X - 5.561$.

The average deviations in percentages of the observed averages are as follows: digestive tube, 4.29; esophagus, 3.62; stomach, 3.35; small intestine, 6.24; large intestine, 5.68; liver, 5.71 and the pancreas, 4.69 per cent.

The formulae for the weights of the female organs plotted on body length from 450 to 560 millimeters are:

Digestive tube,	$Y = 0.52X - 144.954$,
Esophagus,	$Y = 0.028X - 8.976$,
Stomach,	$Y = 0.107X - 33.15$,
Small intestine,	$Y = 0.33X - 92.55$,
Large intestine,	$Y = 0.1X - 31.555$,
Liver,	$Y = 0.3X - 63.551$,
Pancreas,	$Y = 0.04X - 13.93$.

The average percentage deviations for these formulae are: digestive tube, 2.23; esophagus, 1.70; stomach, 1.13; small intestine, 2.24; large intestine, 0.92; liver, 2.34 and the pancreas, 3.95 per cent.

The formulae for the lengths of the digestive tube and its divisions plotted on body length, with both measurements in millimeters are as follows for the male cats from 450 to 610 millimeters:

Esophagus,	$Y = 0.31X + 31.235$,
Small intestine,	$Y = 1.13X + 861.5$,
Large intestine	horizontal line av. 264.98 mm.,
Digestive tube,	$Y = 1.55X + 1222.5$.

The average deviations for these formulae are: esophagus, 2.11; small intestine, 2.37 and for the digestive tube, 1.90 per cent.

The formulae for the linear dimensions of the digestive tube and its divisions plotted on body length in the female cats from 450 to 560 millimeters are as follows:

Esophagus,	$Y = 0.4X - 14.0$,
Small intestine,	$Y = 4.7X - 1070.0$,
Large intestine,	horizontal line av. 250.57 mm.,
Digestive tube,	$Y = 4.77X - 535.0$.

The average percentage deviations for these formulae are: esophagus, 1.62; small intestine, 1.61 and for the digestive tube, 1.15 per cent.

In the above lists of formulae, there are no formulae given for the lengths of the large intestine plotted on body weight and on body length for the females and also for the length of the large intestine plotted on body length in the males. In all three cases the distribution was a horizontal line with only the usual individual variations. In the case of

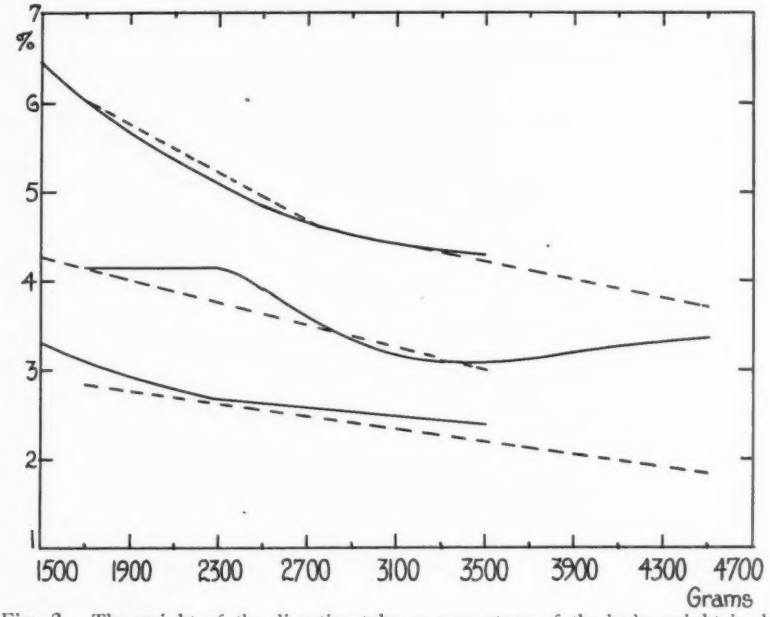

Fig. 2. The weight of the digestive tube as percentage of the body weight is shown in the upper pair of curves. The solid line represents the females and the dotted line the males. The middle pair of curves, with the solid line representing the males and the dotted line the females, shows the percentage of the liver. The lowest pair of curves represents the percentage of the pancreas. The solid line represents the females and the dotted line the males. All curves represent changes in the percentages of the body weight and are plotted on body weight.

the length of the large intestine plotted on body weight, the curve had a very slight slope and yet it was without doubt not a horizontal line. This would indicate that in only this one condition, namely large intestine length plotted on body weight in the male cats, is there any appreciable increase in length of the large intestine in the larger cats. The correlations (Table II, Latimer, 1937) show that the length of the large intestine in the females correlated with both body weight and body length, and in the males, the correlation with body length are not

significant, and the correlation of the large intestine length with body weight in the males is larger but only +0.461. Hence we are justified from both of these methods of study, in saying that the length of the large intestine does not vary with the weight or the body length of the adult female cat, and in the males there is no increase with increase in body length and but a slight increase with the increase in body weight.

The fit of the above formulae is better in the females in all of the 41 formulae except in two cases, or for the esophagus weight and length plotted on body weight. The average deviations of the weights of the digestive system plotted on body weight are 59.8 per cent greater in the male cats and the lengths of the parts of the digestive tube plotted on body weight average 2.17 times greater in the males. The weights in the males plotted on body length exceed the average of the females by 2.31 times and the lengths in the males plotted on body length are 45.68 per cent greater. Both in the digestive system and in the nervous system, the fit of the formulae is on the average better in the females. All of the formulae in the males, both for the weights and for the linear dimensions, are better fitted on body weight than on body length. The same is true for the females except for the weights of the esophagus, the stomach and the liver. Some of the average percentage deviations for the parts of the digestive system are greater than any found in the nervous system. The highest deviation in these formulae for the digestive system is 6.24 per cent, with a second highest of 5.71 and all of the others are below 5.00 per cent. These are not high percentage deviations and so these formulae should give a better prediction of the weights and lengths of the parts of the digestive system from either body weight or body length than the average dimensions.

The weights of the digestive tube, the liver and the pancreas were determined as percentages of the body weight and the individual percentage weights plotted on body weight. Curves were drawn through the averages as was done for the absolute weights. These curves are shown in Figure 2 without any of the individual percentages. All of these curves decrease with increasing body weight, or all of these organs are relatively heavier in the smaller cats. All of these curves with the exception of that for the percentage weight of the liver in the male cats are either straight lines or curves slightly concave superiorly. There was a good deal of variation in the percentage weights of the liver in the males up until about 2300 grams of body weight but with the central tendency a horizontal line. Following this came a decrease in percentage weight as in the other parts of the digestive system, but in this curve alone there was an increase in the larger cats. No doubt this increase in relative weight of the liver in the larger cats was due to the deposit of fat in the liver, as has been suggested for the liver in the older male Leghorn chickens (Latimer, 1924). In gen-

eral it seems that the parts of the digestive system like the parts of the nervous system, are relatively larger in the smaller cats.

SUMMARY

Empirical formulae have been developed for the more accurate determination of the weights of the brain and of its four major divisions, of the pairs of eyeballs, and of the spinal cord, also for the weights of the digestive tube and of its four divisions, of the liver and of the pancreas. Formulae are given for the linear dimensions of the digestive tube and of three of its divisions. All of these weights and linear dimensions may be computed from either the body weight or the body length, within certain limits.

In general, these formulae fit better in the female cats and better for body weight than for body length. The average percentage deviations of the calculated from the observed average values are in all cases comparatively low.

The percentage weights of the brain, eyeballs, spinal cord, digestive tube, liver and pancreas decrease from their maxima in the smallest cats. The liver alone shows a secondary increase in the largest male cats.

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GROWTH, HEREDITY AND ENVIRONMENT

By

SIGISMUND PELLER

Research Associate, Department of Anatomy of the Graduate School of the New York University, New York, N. Y.

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The purpose of this study is to analyse the influence of environmental changes upon physical growth in the prenatal and puberty periods, upon sex maturation and upon mental development. Data were taken from the General Hospital and from the Bureau of Vocational Guidance, both in Vienna. The material is especially suitable for a study of this kind. The World War blockade had more devastating results in Vienna than in other Central or West-European areas; the economic crisis following 1929 was very severe; systematic and continuous studies on conditions of life and on growth were carried out on a larger scale than elsewhere. Nevertheless, the results of these studies, scattered throughout the German literature, were almost unknown to the student of growth (see Jackson, 1925, and Levinne, 1939).

NEONATAL PERIOD

Children born in Vienna from 1916 to 1922 were smaller and weighed less than children born in previous years. This was most manifest in the first *post*-war years. The decline is the more striking because there was a corresponding prolongation of the term of gestation.

The children in this study were divided into: a) children whose mothers were poor and did not receive institutional care in a rest-home attached to the lying-in department; b) children whose mothers were poor but received such institutional care from one to eight weeks prior to delivery because of indigency and not of illness; c) children whose mothers were poor but married; and d) children of the well-to-do class (Table I).

Indigent mothers who spent some time before delivery in the rest-home gave birth on the average to longer and heavier children than mothers of a similar social and economic group who entered the hospital within the last week of their pregnancy. Legitimate children were better developed than illegitimate. Children of wealthy mothers were on the whole both longer and heavier than children of either one of the other groups.

The more time an indigent woman spent in the rest-home, the greater was the average length and weight of her child. Correspondingly, the

gestation period was prolonged, the rate of growth per day was increased and the index weight/length^3 became higher. (Only values of the same dimension may be logically compared. In the index W/L^3, the symbol W represents the average weight which is a three dimensional value, and L represents the average length which is one dimensional. Hence the latter had to be raised to the third power.)

TABLE I

FIRST-BORN CHILDREN IN VIENNA'S "GENERAL HOSPITAL" BEFORE AND AFTER THE WORLD WAR*)

Both Sexes	1910 - 1913				1920 - 1922			
	Number of cases	Average Length in cm.	Average Weight in gr.	W/L^3	Number of cases	Average Length in cm.	Average Weight in gr.	W/L^3
a) children whose mothers entered the "General Hospital" 0-7 days before delivery								
Illegitimate	3188	48.8	3049	2.62	697	47.95	2909	2.64
Legitimate	605	49.75	3145	2.55	820	48.95	3037	2.59
b) children whose mothers were living in rest-home for one to eight weeks before delivery								
Illegitimate	1182	49.9	3249	2.61	268	49.1	3141	2.65
c) children of wealthy families in a private ward	264	50.94	3249	2.46	—	—	—	—

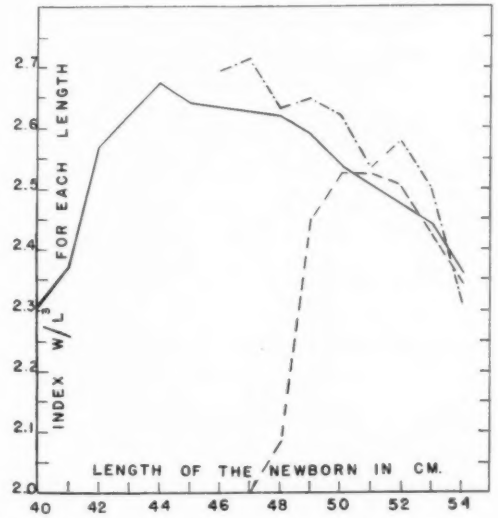
*) In the various studies in which I accumulated measurements of about 20,000 newborns, the probable error (P. E. $= 0.6745 \sqrt{\frac{\Sigma\delta^2}{n(n-1)}}$) had been calculated. According to this experience, the P. E. for a series of 100 firstborn children is about 30 g, for 200 about 18 g, for 300 individuals 15 g, for 400 individuals 13 g, and for a series of 600 to 700 firstborn about 10 g. As to the length of the firstborn, its P. E. in a series of only 100 children is around 0.25 cm, of 300 children around and rather less than 0.1 cm and in a series of 600-700 children in the vicinity of 0.05 cm.

It is to be emphasized that as far as differences in growth are concerned, two factors, independent of one another, are involved: a) the rate of growth within the same duration of pregnancy; and b) the length of the gestation period itself. Thus, taking unmarried primiparae, who gave birth after 280-289 days to male children, and dividing them into those who had rest-home care and those who did not, it was found that in the first group the boys were 50.7 cm long, they weighed 3347 g and had an index of 2.564; while in the other group, the corresponding values were 50.0 cm, 3268 g and an index of 2.614. It is due to the second factor, i. e. to the increased length of gestation, that children of poor mothers receiving rest-home care had also a higher average index W/L^3 than children whose indigent mothers did not have that privilege.

Women living under favorable conditions throughout pregnancy gave birth to children whose average index W/L^3 was considerably *lower* than that of newborns of less privileged strata. Deterioration of general conditions throughout pregnancy as well as an amelioration of

conditions in the last few months of pregnancy are marked by an increase in the average duration of pregnancy and in W/L^3.

Within any social group, the weight/length^3 ratio calculated for each single length (l) of the newborns rises from the lowest length class through a certain class close to and below the average length of the group, then it drops again. We will write this index w/l^3 in order



The index weight/length^3 for each length class of firstborn in three groups of mothers.

— — — — Wealthy mothers, married.
———— Unmarried mothers who entered hospital 0-7 days before delivery.
— · — · — Unmarried mothers who entered hospital 1-8 weeks before delivery.

to distinguish it from W/L^3, which represents a total social group of newborns. Depending on environmental conditions, the class of length with the highest w/l^3 differed more (ranging from 44 to 50 cm) than the average length of the groups (from 48 to 51 cm). Under conditions retarding the growth of the fetus, the newborn reached its highest index at the length of 44 cm; this index being 2.675. In the wealthy group, the highest index was only 2.532, and was to be found in newborns not smaller than 50 cm. The class of the highest index is the only one which, when compared with any other higher class of length, exhibits a constant proportion between increase in weight and increase in length. w_o being the weight and l_o the length of the group of newborns which is characterized by the highest w/l^3, and $\triangle w$ as well as $\triangle l$ representing the increase in each one, then

$$\frac{\triangle w/w_o}{\triangle l/l_o} \equiv 3$$

This is analogous to Gay-Lussac's law expressing the ratio in changes between the volume or pressure of gas and changes in temperature.

Under conditions retarding the growth of the fetus, both significant classes of length, i. e. that of the highest index w/l^3 and that of the average length, become considerably dissociated from each other, and the range of length classes in such a group of newborns is relatively wide (Fig. 1). From this graph one realizes that a newborn 46-48 cm long in the under-privileged series is more likely to be "mature" than in a series of wealthy mothers. In classifying a newborn as premature, the consideration of anatomical signs has to be combined with a consideration of the social status of the mother.

Turning back to Table I, one learns that the extreme differences in the averages due to environmental circumstances were as high as 3 centimeters and 340 grams. Thus they exceeded the difference between boys and girls by about 3 times and the difference between first- and second-born children by more than that. It is therefore not surprising that an environment which does harm to physiological development also influences viability and endangers the life of the fetus (Peller, 1923, 1924).

During the last 40 years, the decline in infant mortality rates has not held true for the neonatal period (0-7 days). In accordance with Ylppö (1919), Schwartz (1922), Holland (1922) and Heidler (1928), the problem of child-death at birth has been connected with cerebral contusions and intracranial hemorrhages, and an increase in qualified medical aid was considered the way to overcome that problem. In these studies the anatomical findings as a cause of death were highly overrated (Ford, 1926; Peller, 1930; Sellheim, 1932; Creutzfeldt and Peiper, 1932; Catel, 1932; Toverud, 1936; Potter and Adair, 1939 and others). The unsolved problem of stillbirth and neonatal mortality is very closely related to the postconceptional factors influencing growth of the fetus and its vigor of life at the end of the intrauterine period. For each length the weight was on the average 380 g higher in children who survived birth than in those who succumbed at or soon after birth. The deficit was higher in small children (512 g. in 39-44 cm and 289 g. in 45-55 cm long newborns.)

The bad conditions of life in many European countries during the first years after the world war impaired the physiological growth of the fetus and by this very fact led to a general increase of the stillborn rate as well as of the neonatal mortality. In the Allgemeines Krankenhaus, Vienna, the stillborn rate plus neonatal mortality for illegitimate children varied between 10.2 and 3.2% depending on

whether the mother had come to the hospital during the last week before delivery or at an earlier date. The corresponding data for the legitimate children were 8.8 and 1.6%. No specific medical treatment had a bearing on this reduction to values never and nowhere achieved, even not in wealthier population groups.

MENARCHE

In 1920-21 in Vienna, according to Rosenfeld (1929) girls between 14 years and 14 years 5 months were on the average 149.3 cm tall; only 26% of them had menstruated. Ten years later, in 1931, an unselected group of 1,126 girls of the same age (measured by me and my coworkers, Dr. Zimmermann and Dr. Berg) was 4.6 cm taller, reaching an average of 153.9 cm; 76% of them had menstruated (Peller and Zimmermann, 1932). In 1920/21, of girls who were one year older than this group (e. g. 15 to 15.5 years), only 64% had passed this stage of maturation. In 1931, girls who had *not* menstruated by the first half of the fifteenth year were shorter by 3.7 cm than those who had, and 6.7 cm taller than a sample of *all* girls of the same age in 1920/1921.

The percentage of Vienna girls who had reached the menarche by the first half of the fifteenth year during 1931 was close to the figures obtained in this country. At the end of the fourteenth year of life, according to Abernathy (1925), 65% reached the menarche, according to Boas (1932) 77 to 78%, and according to Shuttleworth (1937) 86.5%.

As far as the menarche is concerned, in 1931 girls in Vienna were relatively 1.5 years ahead of those of 1921, and also much ahead of those who were born at the turn of the century (Peller, 1935). In 1920/1922, a girl in the first half of the 15th year had still to grow a lot and gained about 3.5 cm during the following twelve months. In 1932, the Vienna girl in the first half of the 15th year (1,125 were measured) had the period of rapid growth behind her (Table IV); she was on the average 154.5 $\pm$ 0.3 cm tall, i. e. 5.2 cm taller than in 1920 to 1922. She also was 0.7 cm taller than a girl of the same age in 1931 (153.8 $\pm$ 0.2), but she was smaller than her sister of the same age in 1933. In 1931, of the 14 years to 14 years 5 months old daughters of the working class 27% had not yet begun to menstruate, while in families of professional businessmen, officials and clerks, there were only 12.5% in this stage of development. At the age of 15 years to 15 years and 2 months, the respective figures were 17 and 5%.

An interesting example of environmental influences upon our physis is the season and the day at which the first menstruation, the menarche, occurs. This was studied in 5,500 girls. Of 100 cases of menarche, 13.4 $\pm$ 0.8 had their first menstruation in January, while on the supposition of a fairly even distribution 8.3 $\pm$ 0.6% were to be expected.

TABLE II
MEASUREMENTS IN 1929
(BUREAU OF VIENNA'S VOCATIONAL GUIDANCE)

Year of Birth	Age in Years	5,544 BOYS						3,160 GIRLS					
		Height			Weight			Height			Weight		
		cm	SE*	cv**	kg	SE*	cv**	cm	SE*	cv**	kg	SE*	cv**
1915/16	13.5-14.0	151.5 ± 0.3		5.4	41.65 ± 0.25		17.0	152.9 ± 0.3		4.2	45.5 ± 0.3		15.6
1914/15	14.0-14.5	153.2 ± 0.2		5.4	42.9 ± 0.15		16.3	153.6 ± 0.2		4.3	46.4 ± 0.2		15.3
	14.5-15.0	155.1 ± 0.2		5.4	44.9 ± 0.2		17.3	154.5 ± 0.2		4.2	47.0 ± 0.2		14.6
1913/14	15.0-15.5	157.7 ± 0.3		5.6	46.8 ± 0.3		16.8	154.6 ± 0.3		4.3	47.6 ± 0.3		14.9
	15.5-15.9	159.8 ± 0.5		5.6	49.6 ± 0.4		16.5	155.3 ± 0.4		4.0	49.0 ± 0.4		13.0

* Standard error, SE
** Coefficient of variation, cv

TABLE III
MEASUREMENTS IN 1929-32*)
(BUREAU OF VOCATIONAL GUIDANCE)

Year of Birth	Age in Years	17.812 BOYS				11.656 GIRLS				BOYS		GIRLS	
		No.	cm	kg	W/L³	No.	cm	kg	W/L³	Gain in cm	Gain in kg	Gain in cm	Gain in kg
1915/19	13.5-14.0	3707	152.7	43.0	1.207	2457	153.6	46.2	1.274				
1914/18	14.0-14.5	6721	153.6	43.8	1.208	4098	153.9	46.7	1.280	0.9	0.8	0.3	0.55
	14.5-15.0	3795	155.6	45.3	1.202	2595	154.2	47.5	1.295	2.1	1.5	0.3	0.75
1913/17	15.0-15.5	2286	157.0	46.7	1.207	1662	154.2	47.8	1.303	1.4	1.4	0.0	0.3
	15.5-15.9	1303	157.8	48.0	1.217	844	154.3	48.6	1.323	0.8	1.3	0.1	0.8

*)This Table is based on 40 pairs of *means* expressing Height and Weight for the age 13.5-15.9 years and the period 1929-32. Grouping these 40 pairs of means into two correlation tables, one gets the following regression equations:

♂ ♂	♀ ♀
H = 1.086 W + 106.5	H = 0.424 W + 134.3
W = 0.909 H — 96.2	W = 1.231 H — 142.3

The correlation coefficient (Pearson) for boys is + 0.994 ± 0.002, and for girls + 0.724 ± 0.073. These coefficients are higher than they would be if all *single* values of the 29,468 individuals instead of the *mean* values were used (see Berkson, 1929).
The standard error, the value σ and the coefficient of variation for all data of each year 1929-31 were given in my publication Klin. Wochschr. 1933 Nr. 17.

Engle and Shelesnyak (1934) showed a similar distribution for the U. S. In Vienna the actual January figure surpassed the percentage of all other months including May* (9.3 ± 0.7%). July, November and December had also a higher frequency of menarche than the average month. During the birth-month, 13.7 ± 0.8% of all girls menstruated for the first time. The Christmas and New Year holidays, birthday parties, the coming of spring, the closing of the school year and the pa-

*According to C. E. Palmer (1939), the rate of weight growth is at its lowest in May and at its highest in October.

TABLE IV

MEASUREMENTS IN 1932

(BUREAU OF VOCATIONAL GUIDANCE)

Year of Birth	Age in Years	3,827 BOYS		2,960 GIRLS		GAIN IN			
		Height cm	Weight kg	Height cm	Weight kg	Boys cm	Boys kg	Girls cm	Girls kg
1918/19	13.5-14.0	152.9	43.4	154.4	46.5				
1917/18	14.0-14.5	154.6	44.7	154.5	47.1	1.7	1.3	0.1	0.6
	14.5-15.0	156.1	45.6	154.1	47.7	1.5	0.9	−0.4	0.6
1916/17	15.0-15.5	157.8	47.7	154.6	49.2	1.7	2.1	0.5	1.5
	15.5-15.9	159.1	49.0	154.5	48.8	1.3	1.3	0.1	−0.4

TABLE V

1,579 VIENNA GIRLS MEASURED IN JANUARY TO APRIL 1933

(BUREAU OF VOCATIONAL GUIDANCE)

Year of Birth	Age in Years	Number	Height cm	Weight kg	Circumference expir. cm	Circumference inspir. cm	GAIN IN Height	GAIN IN Weight	GAIN IN Circumference expir.	GAIN IN Circumference inspir.
1919	13.5-14.0	480	154.9	47.9	75.8	82.5				
1918/1919	14.0-15.0	922	155.6	48.4	77.0	83.1	0.7	0.5	1.2	0.6
1917/1918	15.0-15.9	177	155.9	50.3	77.2	84.4	0.3	1.9	0.2	1.3

tron day-month, in short, events bringing joy and excitement release the first menstruation in bodies which have reached physiological maturation and wait only for a psychical stimulus.

GROWTH DURING PUBERTY

Generally speaking, growth at puberty is much less studied than at earlier ages. Usually the number of individuals measured in their 14th to 16th year of life is very small. In the following tables, data for 17,812 boys and 13,235 girls between 13.5 and 15.9 years of age are given. These children were measured by me and my collaborators, Dr. Berg and Dr. Zimmermann, during the four years 1929 to 1932 and at the beginning of 1933. The measurements were recorded to the nearest 0.5 cm and 100 g. All children were examined in the morning hours, wearing underwear only. The date of the birth-certificate and the exact age at the time of measurement were recorded. The detailed distribution according to length and weight has been published in the annual reports of the Bureau of Vienna's Vocational Guidance.

The vast majority of these children belonged to the economically under-privileged strata of the Vienna population. They were an unselected representative sample. (The whole population of this age was about $2\frac{1}{2}$ times as large.)

However sensitive to environmental changes the growth during puberty age may be, it did not reflect the growing economic depression.

On the contrary, boys and girls alike were taller and heavier in 1932 and 1933 than in 1929. In Hagertown, Maryland, in the years of depression, Palmer (1933, 1934) also did not find any consistent decline in weight for the age groups 6 to 11 years, but his figures did not show an increase in weight.

The explanation for the increase in length and weight in Vienna is not to be sought in this very period (1929-32), but in the general conditions prevailing some 15 years earlier when these 30,000 individuals were either unborn or in the first years of life. In other words: the figures presented above are an example of the physiological problem of recovery from chronic undernourishment thrust upon normal and healthy fetuses and children.

Many studies have dealt with the problem of whether or not animals and plants are capable of full recovery after a period of acute and extreme inanition, or after alternating inanition and refeeding. According to Jackson (1925), inanition at a very early stage of extra-uterine life is a more serious drawback than when started at a later period.

As to human beings, there exist more observations on diseased, severely undernourished children, than on children moderately yet chronically undernourished for socio-economic reasons (Aron, 1914; G. Wolff, 1932). Our data enabled us to study the *remote* effects which underfeeding during fetal life and during the first years after birth has upon adolescence. Hence we were studying the effect of malnutrition in a period separated from the time of scarcity of food by a long series of years of adequate food supply.

Vienna's population reached the pre-war level of nutrition (Peller, 1919) during 1925 and passed it in the following year. According to my studies (1928, 1931, 1934), carried out together with B. Kautsky (1937) in 1926 to 1928, an adult male of the working class in Vienna consumed 3,191 calories, 92.5 grams protein and 125 grams of fat. During 1932 the average number of calories was 3,038, of protein 85.7 grams and of fat 118.5 grams.

These values were obtained from the daily registration carried out during all the years 1926 to 1932 in 118 families carefully selected and regularly supervised by a staff of field workers of the Arbeiterkammer. The nutritional value of the consumed articles had been analyzed by the Physiology Department of Vienna University. A number of other families supplemented the study with daily data for at least one year.

In these years, even the unemployed tried to maintain the nutritional standard, exchanging and substituting cheaper products for expensive food. That was possible. In 1929 the price per 1000 calories was rising directly with the income and inversely with the size of the family from

X to 1.54 X. In 1932, in the families of unemployed, the daily average for an adult male was 2597 calories, 65.9 g. protein and 88.8 g. fat. Although during the years 1929-1932 the nutritional level had been slowly dropping, the average values of growth improved from year to year. That is one side of our experience. On the other side, one learns that the damage done to children by environment during intra-uterine life or in the first years of life (after birth) or at both times overshadows their life for a long period.

Comparing our values with those for the 14 year old boys and girls in U. S. A. as reported by Schwartz, Britten and Thompson (1928), by Collins and Clark (1929), and by Gafafer (1936), one has to conclude that the consequences of the condition of want during 1916 to 1922 were still marked in the growth of Vienna's adolescents during 1929 to 1932. A span of about 10 to 11 years hardly suffices to repair the damage done to the fetus and infant. The 14 year old "old American" boy (155.5 cm and 45.1 kg) and girl (155.5 cm and 46.75 kg) were taller and heavier than Vienna's children of the same age in 1932, but in 1933, for girls, the difference in length diminished to 0.5 cm, while for the average weight, the difference entirely dissappeared. The Vienna girl by 1933 had a higher height-weight than the American girl as reported in the studies of the U. S. Public Health Service. It may be due to this fact that the Vienna girl had also a higher chest girth, the difference being about 5.9 cm (American girl between 13.5 and 14.5 years of age: 28,845 inches =73.3 cm, as compared with 79.2 cm in Vienna). Differences in the average age of sexual maturation did not account for this.

The studies of the Public Health Service gave lower values for height and weight than Gray and Ayres (1931) found for wealthy children in a private school in California. The 13 to 15 year old boys and girls of this school were 9 to 12 and 5 to 7 cm respectively taller than corresponding adolescents of the lower strata in Vienna in 1932 or 1933. The differences seem to be statistically significant regardless of whether Vienna's youth is compared with "old-American" stock or with Jewish-American youngsters.

According to Gray (1927), American boys of native stock are now 2 inches taller than 50 years before; according to Bowles, Gordon, Townsend (1932), "old Americans" gained 7.5 cm in 100 years. Similar gains have been observed in Europe. Half a century is a very long time for changes. We, however, deal with a period of 4 years only. According to our tables, in 1932, the 13.5 to 14.5 year old boy was 1.4 cm taller and 1.8 kg heavier than a boy of the same age in 1929; and for a girl the difference was 1.3 cm and 0.9 kg. These changes were due to the reactions of growing bodies to environmental conditions

which were about equal at the time of the measurements, but different 8, 10 or 14 years earlier.

Another example of environmental influences is the relationship between size of family and development of the child. In 1926-1928, with rising size of the family from less than 2.0 to over 4 consumption units, the number of calories per unit declined from 3472 to 2972, the amount of protein from 104.6 to 82.5 g and of fat from 155 to 110 g. (In former times the respective variations were still greater). It is not surprising that such differences have a bearing on the child's growth. The best developed children are those who have one sibling, the second place is taken by the only child, then follow children with 2, 3, 4, and more siblings. This may be exemplified in 10,516 boys in the fifteenth year of life. In intact families*) with 1, 2, 3, 4, 5 and more children, the average height of these boys was 155.2, 156.4, 155.0, 153.2 and 151.9 cm, respectively; the average weight: 45.6, 46.5, 44.6, 42.6 and 42.5 kg, respectively (Peller and Bettelheim, 1933). The differences range up to 4.5 cm and 4 kg. The findings for boys of other age groups and for girls as well were analogous.

Dividing the group of 704 orphaned boys who were 13.5-15.9 years old and of tuberculous parents, into 3 groups, a) orphaned below 6 years of age, b) between 7 and 10, and c) over 10 years, the average length was 155.4, 154.8, and 152.9 cm, respectively. The average weight was 46.0, 44.3, and 42.7 kg, respectively**. Similar results have been obtained for 506 girls.

The parents' deaths, regardless of the cause of death, left its imprint in the maturation of girls and in the scholastic achievements of both sexes. In 1931 and 1932, in intact families, girls in the 15th year of life had menstruated in 80.6 $\pm$ 0.6%, whereas orphans of the same social group and age only in 70.8 $\pm$ 1.8%.

MENTAL DEVELOPMENT AT PUBERTY

Still another point may be briefly mentioned although it is not concerned with physical growth, namely mental growth. This depends on heredity and environment just as much as physical growth does. In our study we used school grades, school achievements to indicate endowment. We were working with so large a group (over 30,000 youngsters) that measurement of the Mental Age was not feasible, although desirable. Recent studies, in the first place the "Iowa Studies" carried out by Wellman (1940) and Skeels (1940), indicate that Intelligence Quotients measure innate intelligence plus exposure to educational opportunities of the formal (school) and informal type.

*) Father and mother alive.

**) To all these groups, the age distribution of the total of 17,812 boys had been applied.

The last factor, i. e. the level of foster-family and its willingness to "foster" (in the specific sense of the word) the child seems to outweigh the "true-family-background" and the innate intelligence. It is also probable that the results obtained by elaborated Intelligence Tests do not differ substantially from results gained with much simpler devices such as the vocabulary part of the test.

Of the children of intact Viennese families, (818 *un*selected cards of a few files were studied) 9.5 ± 1.0 per cent exhibited good and 22.4 ± 1.4% showed unsatisfactory school achievements. For 2,699 orphaned children of the same age composition, the respective figures were 5.4 ± 0.4 and 33.9 ± 0.9 per cent. The difference between orphans and non-orphans cannot be correlated with different distribution of innate talents in the respective groups of children. Neither are they due to physical handicaps. They are due to differences in the environmental conditions of life. This is analogous to the different frequency of delinquent children in families with divorced and not divorced parents as shown by C. Burt (1925).

Comparing orphans with non-orphans, there is a wider gap between the school achievements in girls than in boys. This difference is most likely due to the greater share of household duties that are the girl's lot in bereaved families. In school achievements, girls who lost their mothers are below girls whose father died. That again reminds us of the results obtained in Burt's study on delinquents.

SUMMARY AND CONCLUSION

Among the factors determining *a*) the rate of fetal growth, *b*) the duration of pregnancy, and through both *c*) the final length, weight, the weight:length^3 ratio of the fetus, and time of birth, those due to the conditions of life of the pregnant mother play a most conspicuous role.

Rest homes for expectant mothers bring about a definite and extensive change in all the features just mentioned and a not otherwise achieved reduction in neonatal mortality and stillbirth rate, even for children in the lowest strata of society.

The influence of prenatal conditions upon growth and development of the child is of even longer duration. Unfavorable conditions for mothers during the intra-uterine period and for children in early childhood leave their imprint for 13 to 15 years, in spite of favorable conditions during a later, essential part of the period of growing.

During 1929/33 the weight and length of more than 30,000 boys and girls, then 13 to 15 years of age, were studied and compared with children of the same city (Vienna) in 1921, and with American youth. Further, for 1500 girls the chest-girth was compared with American values. A decade of more favorable conditions resulted in only *gradual*

repair of the damage done to the fetus and small child by war and post-war conditions. Therefore during 1929/33 the average values rose from year to year. They rose in spite of the economic depression and its consequences upon the standard of life.

Sex maturation was studied in girls and found to be linked not only with their physical development and the social status of the parents, but also with psychic events (birthday-parties, Christmas, festivities, etc.).

School achievements of orphaned children were compared with those of children from intact families, and were found to be impaired.

Nature determines what a human being may be, nurture what he actually becomes. Nature sets potent though phenotypically invisible barriers. Within these limits nurture sets the pace.

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A COMPARISON OF THE ENERGY-RELEASING ORGANS OF THE WHITE WHALE (DELPHINAPTERUS LEUCAS) AND THE THOROUGHBRED HORSE "EQUIPOISE"*

By

GEORGE CRILE

Cleveland Clinic Foundation

and

DANIEL P. QUIRING

Cleveland Clinic Foundation and Western Reserve University, Cleveland, Ohio

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INTRODUCTION

For some time we have concerned ourselves with a study of the energy-releasing organs and glands of the animal body, and in connection with this study we have attempted to evaluate the effect of environment as it is represented by such factors as temperature and habitat. By energy-releasing organs we refer particularly to the brain, the thyroid gland and the adrenal glands. Considering the differences in the environmental factors to which the thoroughbred horse and the white whale are normally subjected, they should make ideal objects for study.

The purpose of this paper, then, is to compare certain organs of the white whale with those of the thoroughbred horse. Through the courtesy of Major L. A. Beard of the Whitney Stables at Lexington, Kentucky, we were able to secure glands and organs and make a partial dissection of the body of the famous thoroughbred racing stallion, Equipoise. This animal weighed 521.51 kilograms. In the summer preceding, we killed six white whales in the Churchill River Inlet on Hudson Bay, Canada. It so happened that one of these animals matched Equipoise, to the kilogram, in body weight. The body weight of this male white whale was 521.18 kilograms.

CHARACTERISTICS OF EQUIPOISE AND THE WHALE

Equipoise traces his descent from one of the three great horses primarily responsible for the English thoroughbred. This was the English horse Eclipse (1764). Eclipse in turn represents the result of

*This description, while based on two animals has as a background a study of six white whales and 133 thoroughbred horses. Since the two animals described exemplify the outstanding characteristics of the species, and because they were so equally matched in body weight, it was hoped that such a description would be more effective than a more extensive one, in demonstrating differences between their glandular mechanisms.

the crossing of the English mare with the Turk, the Barb and the Arabian horse. The English mare was derived from the English forest pony, while the origin of the Barb, Turk and Arabian is shrouded in the past.

Equipoise was an outstanding horse and his racing record was phenomenal. At the close of his second year, his winnings totaled $156,835; in his fourth year he won $107,375. He continued to race through his sixth year. According to J. A. Estes, editor of the "Blood-Horse", "he won 29 out of 51 starts, was disqualified after finishing first on three occasions, and once lost his rider. He earned a total of $338,610, making him second to Sun Beau among the world's leading money winners. The highest weight he carried in competition was 135 pounds and there were 13 occasions when he carried 130 pounds or more." His racing weight was 1,000 pounds and he stood just under 16 hands high. At the time of his death on August 4, 1938, he weighed 1150 pounds. He was born on May 1, 1928, bringing his age to slightly beyond 10 years.

The animal died from a digestive disturbance without preliminary illness. His body and glands showed no pathological changes commonly observed in sickness of long standing.

The white whale, also known as the Beluga whale, was killed by us on August 10, 1937. The color of the animal is a pure milky white. It is probably derived either from Mesozoic insectivores or from ungulate or carnivore-like mammals. It appears most closely related to the porpoise and the dolphin. The most interesting external characteristic is the dorsal blow-hole which is the opening of the breathing tube. This has moved into the dorsal position with the telescoping of the skull (Kellogg, 1928) and has become completely separated from the mouth cavity. This migration of the breathing tube has brought about a curious foreshortening of the brain. Other striking external features are, of course, the absence of external pelvic structures, the great horizontal tail fluke, the position of the mammary glands which lie just lateral to the anal aperture and are covered by folds of the integument. The melon, giving the head an intellectual cast, is the rounded mass of blubber on the dorsal head surface.

Internally, the most striking characteristics are the long intestinal tract and the high degree of vascularization of the thoracic body wall. This vascular network forms a giant rete mirabile and continues as a closely woven net, lining the neural canal which apparently supplants the carotid circulation and supplies the brain through this route. The body musculature is a deep reddish-black in color, indicating the large amount of blood in the tissues. Covering the muscular mass is a layer of blubber, varying in thickness from three to six inches.

We see here two outstanding specimens of two great species which represent the end effect of a long evolutionary development. The one shows the profound effects of natural selection upon structure and energy release, while the other exhibits modifications produced by artificial selection upon a naturally selected stock. Both of these animals are characterized by great energy; the one has to spend energy constantly by virtue of his sea life and his incessant need to come to the surface for breathing; the other has great intermittent energy expenditure forced upon him by man. Let us see how they compare in the degree of development of energy-releasing organs. The heart is included in these data since it reflects faithfully the energy habits of the animal. It represents the servant while the systems noted above (brain, thyroid gland and adrenal glands) are the master. The following table shows the comparative standing of the two animals in weight and ratios of these parts to the body weight.

	Body Weight	Brain	Thyroid Gland	Adrenal Gland	Heart
Equipoise	521.51 kg.	808.5 g.	33.40 g.	46.62 g.	4455 g.
	Ratios	1:645	1:15,614	1:11,180	1:117
White Whale	521.18 kg.	2355 g.	108 g.	34.76 g.	3181 g.
	Ratios	1:221	1:4825	1:15,328	1:194

BODY WEIGHT

The body weights of the two animals are probably close to the average for the two species. As a matter of fact, the racing weight of the thoroughbred may be from 100 to 150 pounds lower. The white whale may reach a weight of 1400 to 1800 pounds according to information given us by northern trappers. Such weights are probably the exception, for we had abundant opportunity to observe large schools entering Churchill Inlet and we saw few specimens that appeared much larger than the object of this study.

THYROID GLAND

The thyroid weight of Equipoise was 33.40 grams. This appears typical for the thoroughbred horse in which ratios from 1:15,000 to 1:20,000 are common. In comparison with the white whale, where the gland weighed 108 grams and the ratio is 1:4825, the horse gland is small and apparently reflects the difference in metabolism requirements of the two bodies. In one of our whales, the thyroid ratio was 1:2842. We have found a large thyroid gland to be characteristic of northern animals, of completely aquatic mammals whether seals, walruses, porpoises, or whales, and of man. In no other group of

comparable weight have we observed such large thyroid gland ratios. Man forms the only exception to this rule. When it is considered that the white whale lives in water which remains within a very few degrees of freezing (0°-8° C.) the year round, and that frequently it is required to negotiate considerable distances under ice, this high thyroid ratio is understandable, since we must look to the thyroid gland to establish the basal metabolic rate.

The histology of the thyroid gland also shows interesting differences when compared to the normal horse thyroid gland. The gland of the white whale appears hyperplastic, the epithelial cells are tall, cylindrical in shape, vacuoles are numerous, the colloid secretion shows serrated margins, the vascular supply is more profuse than in the horse. Since all of the whale thyroid specimens show similar histological pictures, we assume that this is the normal state of the gland. The horse thyroid in section exhibits low epithelial cells with considerable amount of colloid substance present, indicating a normal quiescent condition.

In spite of a heavy blubber insulation against heat loss, such losses must be considerable as Townsend (1914) observed in transporting five porpoises from Cape Hatteras to New York. He found it necessary to change the water every five or six hours because of the heat radiated from the animals' bodies. Presumably, the large thyroid gland is a response to these conditions.

ADRENAL GLAND

The adrenal gland weight was 46.62 grams for Equipoise, in comparison with 34.76 grams for the white whale. We estimate that about 15 to 18 per cent of the gland in both animals accounts for the medullary portion. It is possible that the constant activity of the white whale makes relatively lesser demands upon this gland, and higher demands on the thyroid gland than is the case in the horse where great outbursts and rapid mobilization of energy are the rule and consequently appear to affect adrenal gland size. This may appear as an acceptance of the theory of use and disuse but this is not implied. We have observed for example that the weight of the adrenal glands of two male lions shot by us in the African jungle was just over twice that of three male captive zoo specimens, and we attribute these differences to differences in the habits of the two groups. In the case of Equipoise and the whale, inherited differences are, of course, not ruled out.

The adrenal-thyroid ratio in the whale is comparable to that in man. Again we find such a ratio characteristic in the northern aquatic mammals and in man. In other animals, only the rare exception shows thyroid gland dominance.

BRAIN

The brain of Equipoise, which weighed 808.5 grams, is the largest we have found in any horse and we have examined over 133 specimens. It is heavier by 73.5 grams than the next horse brain, which is that of Pennant, sire of Equipoise. It is 190 grams heavier than that taken from a pure Arabian stallion of comparable weight. This brain is characterized by the great breadth of the parietal portions of the cerebral hemispheres, by a large cerebellum, and by its length. According to Simons, the cerebellum comprises about 9 per cent by weight of the entire horse brain. It appears obvious in comparing the thoroughbred brain with that of other breeds, that the breeder has been selecting for brain whether he knew it or not and that the brain apparently summarizes and accurately reflects the energy characteristics which the breeder desires.

The brain of the white whale weighed 2355 grams. As already indicated, it is foreshortened, due to the position of the nasopharyngeal tube passing upward from the larynx immediately anterior to it and opening on the dorsal surface of the head. This is a common cetacean characteristic as Kellogg (1928) and Langworthy (1932) have pointed out. The brain is characterized by great cerebral and cerebellar development. The cerebellum comprises about 18.95 per cent by weight of the cetacean brain according to Kappers (1936). Its convolutional pattern is of greater relative depth and complexity than is that of man. Motor representation apparently is large. Riese (1927) and Langworthy (1931) have pointed out the great adaptive development of such nerves as the acoustic, but this is hardly sufficient to account for the great increase in brain weight. This gain must be counterbalanced by loss of olfactory, and degeneration of visual and general sensory functions.

It is necessary, then, to seek other factors to account for this great brain size. We have approached this problem from a new angle; we have sought for a possible functional relationship between basal metabolism and brain weight to account for brain size. On purely theoretical ground such a concept does not appear illogical, for, reduced to its fundamentals, it makes use of the following facts and implications:

a. Muscle and nerve are functionally connected; if this connection, whether sensory or motor, is broken, the metabolism of the muscle falls, and the tissue atrophies.

b. The nerve passing to the muscle has representation in the cord and in the intercalated cells of the brain which in turn make synaptic junctions with sensory nerves.

c. Since this constant communication is necessary to maintain metabolism, it follows that brain size must be related to basal metabolism.

We have put this concept to the test in both warm and coldblooded animals and find as a result of 860 metabolism tests on 197 warm and coldblooded animals that a very definite correlation apparently exists between brain size and basal metabolism. This relationship is such that at 20° to 24° C. temperature, for every gram of brain substance 12.11 ± 0.419 Calories are produced in 24 hours in animals tested by us, exclusive of man and the primates; while the standard deviation is large, the trend toward this value is unmistakable. We have not determined the metabolism of the white whale, but the great food intake of the porpoise, a close relative, should give a clue to the amount of available energy of these cetaceans. According to Townsend (1914) five porpoises weighing about 300 pounds each, in transport to New York, consumed about 90 pounds of herring and cod per day or about 18 pounds each. Such an amount would have a value of about 15,500 calories per animal or about 108 calories per kg. per animal per day, in comparison with about 53 calories per kg. for man. Such a porpoise would have a working metabolism equal to that of a 1200 pound horse.

If the size of the brain is correlated with heat production, while the surface area determines heat loss, it would appear that the ability to dissipate heat is the determining factor in brain size in warmblooded animals. Therefore, an animal living in a most favorable environment for great heat loss should also possess the largest brain. It has been pointed out by Kellogg (1928), Ries and Langworthy (1937) and others that water is about 27 times as effective as air in facilitating heat loss. Consequently, on the basis of this viewpoint, the whale, the porpoise, the walrus, and the seal can afford a larger brain than is possessed by land animals of comparable weight. This implies that in addition to temperature regulation centers in the brain, the brain as a whole is intimately concerned in the maintenance of body temperature.

In comparison with this great whale brain, the brain of Equipoise appears small indeed, since it is but slightly over one-third its weight. The horse faces the problem of heat dissipation just as does the whale, but the horse is living in a much less effective medium for the dissipation of heat. If the internal heat developed during great energy release cannot be dissipated sufficiently fast, heat stroke develops. Heat stroke then apparently is determined by surface area, i. e., by the effective heat-radiating surface and this in turn determines brain size. In comparing the small with the large members of a species, this is clearly shown, for in advancing from the small to the large animal, the brain increases in size as the body weight raised to the 5/9 power, while the surface area in these same animals increases as the body weight raised to the 6/9 power. In other words, surface area and brain weight increase at closely similar rates.

HEART

The heart of Equipoise weighed 4455 grams, that of the white whale weighed 3181 grams. The whale heart was only 71 per cent as heavy as the horse heart. Heart weight is affected by individual training, by inheritance, and by the load forced upon it in body support. Other factors being equal, the ambulatory animal would have a larger heart than one supported by water. Hermann (1929) is of the opinion that the thoroughbred has a larger heart to begin with than the "regular run of horses", and that it has great capacity for hypertrophy. Howell (1930) quotes Wislocki to the effect that the heart of *Tursiops* is larger than that of an ox. Our data on steers taken from government tables give an average heart weight of 1898 grams for 71 steers with an average body weight of 536 kg. This appears to bear out Wislocki's findings and serves to emphasize the great development of Equipoise's heart.

Only the heart and the adrenal glands of the structures compared are larger in Equipoise than they are in the white whale. This finding appears significant in the light of the difference in energy habits of the two animals. The whale, having solved the problem of body support, may propel himself through the water with minimum energy spent for locomotion unless he travels at a speed high enough to cause turbulence.* The remainder may be utilized for maintenance of body warmth. The horse must carry his own body, but he lives in a medium which does not dissipate his body heat at anything comparable to the rate at which it is lost from a warm body in the sea.

SUMMARY

A comparison of the brain, thyroid gland and adrenal glands and heart of a white whale (*Delphinapterus leucas*) and a thoroughbred racing stallion, Equipoise, showed the following:

a. The thyroid gland of the whale is 3.23 times as large as that of the horse. This great size appears to be an adaptation to maintain the metabolism in the face of great heat loss.

b. The brain of the whale is 2.9 times as large as that of the stallion. This great size is presumably related to metabolism.

*)J. Gray (1936) has made calculations on the horse-power required to propel a 200 pound dolphin and a 53 pound porpoise. On the assumption that the resistance of the water to the bodies would be similar to that encountered by an inert body, he calculated 14 horse-power would be required to move the 200 pound dolphin at a speed of 20 knots. When models were made to simulate the motion of the fins, he found "if the flow of water past the body of a dolphin is free from turbulence, the horse-power developed per pound of muscle agrees closely with that of other types of mammalian muscle."

Krogh estimates a 27 meter blue whale travelling at 10 knots produced 46.8 horse-power based on a drag coefficient characteristic of turbulent flow.

c. The adrenal gland of the horse is 1.3 times as large as that of the whale. This difference in size appears related to difference in energy habits in the two compared forms.

d. The heart of Equipoise is 1.4 times as large as that of the whale. The heart reflects the load carried by the animal, and the training and inherited patterns.

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THE INFLUENCE OF CYSTINE DISULFOXIDE ON PROLIFERATION OF NOSTOC MUSCORUM

By

LEE WALP

The Marine Experimental Station of The Lankenau Hospital Research Institute and Marietta College[1]

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Recent work has shown that sulfhydryl (-SH) stimulates proliferation of blue-green algae (Hammett and Walp, 1939). This is true for a great variety of other organisms. It has further been demonstrated that partially oxidized derivatives of the SH group act as retardants of cell increase in number (Hammett, 1930). Since the Myxophyceae are stimulated by SH, the purpose of the present study was to see if they are also affected by the retardative side of the SH-SO equilibrium.

Nostoc muscorum Born. & Flah. was used. This species had been called *Anabaena sp.* in the previous work. Dr. Francis Drouet corrected the mistake in identification. The materials and methods were similar to those described elsewhere (Walp, 1939, and Hammett and Walp, 1939).

In addition to the regular Bristol's solution, three additional culture media were used in order to obtain a pH range from 5 to 9. In all experiments run in Bristol's with the exception of experiment I, the media were diluted to half strength with distilled water. Both the full- and half-strength Bristol's gave a pH of ±4.8. An acid medium of pH ±6.0 was made as follows: 0.40 g. KH_2PO_4, 0.058 g. K_2HPO_4, 1.0 g. $NaNO_3$, 0.2 g. KCl, and 1000 cc. distilled water. Two basic media were made; the first of pH ±7.8, contained 0.55 g. K_2HPO_4, 0.02 g. KH_2PO_4, 1 g. $NaNO_3$, and 1000 cc. distilled water; the second of pH ±8.6, contained 0.58 g. K_2HPO_4, 1.0 g. $NaNO_3$, and 1000 cc distilled water. All the media were used with and without a trace of iron with no change in result. The KCl was added to the acid medium for K equivalency.

Cystine disulfoxide was added to the tests to give a concentration of 3.2×10^{-7} or 6.4×10^{-7} mg. S as SO. The media were prepared and changed thrice daily. The highly purified cystine disulfoxide was prepared at The Lankenau Hospital Research Institute by Dr. Gerrit Toennies.

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The Table contains the results of the experiments arranged according to pH. It gives the total number of control and test cells for the initial and terminal periods in each experiment, the percentage increase, and the difference between the test and control percentages.

The data indicate three trends: Cystine disulfoxide:

1. retarded proliferation of Nostoc at pH ±4.8.
2. accelerated it at pH ±7.8 and ±8.6.
3. and had essentially no effect at pH ±6.0.

TABLE I

INFLUENCE OF CYSTINE DISULFOXIDE ON CELL PROLIFERATION OF NOSTOC MUSCORUM

Exp No.	Hrs Run	Cont	Test	Total Number of Cells Control Start	Control End	Test Start	Test End	Percentages Increase Cont	Increase Test	Difference (T−C)
				Acid Medium, pH ±4.8						
1	24	9	9	2185	4196	2800	5195	92.0	85.5	−6.5
2	48	7	8	2397	2663	2477	2615	11.1	5.6	−5.5
3	48	6	6	1886	4461	1886	3591	136.5	90.4	−46.1
4	72	7	7	1756	3353	2214	3906	90.9	76.4	−14.5
				Acid Medium, pH ±6.0						
5	72	10	9	1753	1880	1701	1847	7.2	8.6	+1.4
6	72	10	10	1671	1723	1625	1723	3.1	6.0	+2.9
7	72	8	8	631	667	522	565	5.7	8.2	+2.5
				Alkaline Medium, pH ±7.8						
8	48	10	10	611	633	619	784	3.6	26.7	+23.1
9	72	6	6	670	723	612	738	7.9	20.6	+12.7
10	48	9	9	982	1107	703	826	12.7	17.5	+4.8
11	48	7	7	634	767	707	969	21.0	37.1	+16.1
12	72	6	6	479	523	503	597	9.2	18.7	+9.5
13	72	5	8	540	643	587	735	19.1	25.2	+6.1
				Alkaline Medium, pH ±8.6						
14	48	9	10	1055	1346	1876	2849	27.6	51.9	+24.3
15	72	7	5	732	814	473	550	11.2	16.3	+5.1
16	48	9	8	1940	2339	1629	2363	20.6	45.1	+24.5
17	48	5	5	663	932	779	1318	40.6	69.2	+28.6

DISCUSSION

There is no question that cystine disulfoxide under appropriate conditions acts to retard Nostoc proliferation. There is also no question that it can stimulate the phenomenon. The direction of influence is apparently determined by the acidity or alkalinity of the culture media. This is nothing new, for Hammett (1930) using root tips; Wilson (1939) using Drosophila larvae also found reversal of effect due to shift in hydrogen ion concentration from the acid to the alkaline side. But these workers found acceleration in acid and retardation in alkaline media. It was also found that retardation occurred in Obelia (Hammett, 1933, and Lavine, 1935) and Pagurus (Hammett and

Hammett, 1932). These experiments were done in sea water of alkaline pH.

What is it that causes Nostoc's reaction to be the opposite: viz. increased rather than decreased proliferation in alkaline media, and decreased rather than increased proliferation in acid? Apparently a new factor has been introduced,—a factor which upsets the normal equilibrium of hydrolysis of the disulfoxide in acid and alkaline media. The most obvious new factor introduced into the experiments is chlorophyll. In none of the previous work has this been present. Chlorophyll is a powerful reducing agent. It would seem probable then that in alkaline cultures of growing Nostoc, chlorophyll so acts to reduce sulfoxide that in the resultant equilibrium SH rather than SO is dominant.

This assumption was put to the test. Two large lots of Nostoc were incubated in Bristol's media at room temperature with ample cystine disulfoxide for 24 hours: one in 4 cc. acid medium at pH 4.8, the other in 4 cc. alkaline medium at pH 7.8. Two other lots were simultaneously incubated in the same media but without the disulfoxide. Blank controls were run with cystine disulfoxide in the acid and alkaline media without Nostoc. After incubation the tubes and contents were centrifuged. Nitroprusside tests for SH were then made on aliquots of the clear supernatant liquid (Hammett and Chapman, 1938). The results were illuminating. The solution from the Nostoc-disulfoxide *alkaline* culture gave a markedly positive reaction for SH which was persistent. The solution from the *acid* Nostoc-disulfoxide culture gave a very faint positive SH test which faded almost immediately. The solutions from the acid and alkaline Nostoc cultures without disulfoxide were negative for SH. The acid solution of disulfoxide without Nostoc gave a fair test for SH which faded rapidly. The alkaline counterpart gave a much fainter test which disappeared almost immediately. From all the several simultaneous comparisons which were made, it became evident that Nostoc in alkaline culture media reduced the SO of cystine disulfoxide to SH and tended to hold it there to definitely greater degree than in acid media. It can therefore be concluded that SH is predominant in the SH-SO equilibrium of alkaline Nostoc-cystine disulfoxide mixtures, while SO predominates in the acid. This is attributed to the differential reducing action of chlorophyll on the disulfoxide in alkaline and acid media. It would be interesting to extend these observations to other chlorophyllous organisms such as Euglena and multicellular plants. Perhaps differences may be brought out since Inman (1940) finds more chlorophyll *a* in blue-green algae.

The results at pH6 are not surprising since two other closely related

experiments have been reported with similar results. Allison, Hoover, and Morris (1937) also using Nostoc muscorum, found that hydrogen ion concentration was a limiting factor. Even time did not alter their results since their experiments ran 14 days. They report that growth is usually best at pH7.0 to 8.5, decreases in going from pH7.0 to 6.0 and growth initiation stops at pH5.7. They do not report results for as low as pH4.8.

In his root-tip studies, Hammett (1930) also found that in the middle pH range between the effective acid and the effective alkaline concentration there was an indeterminate proliferation reaction. He pointed out that this was to be expected since the SH-SO equilibrium potentiality and its resultant redox properties would be neither that of the acid side nor that of the alkaline, but a mixture of the two. Consistent with and confirmatory to this early work and interpretation, are the findings with Nostoc, for here, too, no essential proliferation reaction to disulfoxide is found in the middle pH ±6.0 range. There is acceleration only when SH is predominant: retardation only when SO is in the ascendant.

Finally, the results here recorded add another organism to the long list of those which demonstrate and illustrate the regulatory influence of the SO-SH equilibrium with respect to increase in cell number. They also show that a formed nucleus is no more necessary for retardation by SO than it is necessary for acceleration by SH.

SUMMARY AND CONCLUSION

Seventeen experiments with *Nostoc muscorum* show that cystine disulfoxide acts to retard proliferation of this blue-green alga in acid media; to accelerate it in alkaline media; and has no essential effect in the middle pH range.

The accelerating effect is attributable to the reducing properties of chlorophyll on the compound in alkaline media: properties which are but little expressed in acid media. Interpretative discussion in terms of SO-SH equilibria and their relation to proliferation are given in the text.

The conclusion is that SH-SO equilibrium is a factor in the regulation of cell increase in number of non-nucleated organisms as it is in those possessed of a formed nucleus.

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CHANGES IN THE SKELETAL TISSUES OF MICE FOLLOWING THE ADMINISTRATION OF THYROXIN*

By

MARTIN SILBERBERG and RUTH SILBERBERG

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine, St. Louis

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In immature guinea pigs, administration of thyroid hormone stimulates, for a certain length of time, the proliferation of the cartilage of the growth zones and of the joints. It also accelerates the differentiation of the proliferating cartilage cells into cartilage cells of the hypertrophic type, and increases the resorptive processes in the osseous tissues (Silberberg and Silberberg, 1938 and 1940). We noticed, however, that the maximum of proliferation was reached after twenty days. It seemed, therefore, desirable to investigate the effect of prolonged administration of thyroid hormone on cartilage and bone.

For this purpose, we studied the changes in the long bones and joints of mice which had received injections of thyroxin in experiments undertaken for other purposes by Leo Loeb.

MATERIAL AND METHODS

Sixteen mice, thirteen of which belonged to strain D, two to strain C57, and one to strain Old Buffalo, received subcutaneously 0.1 mg. thyroxin (Schering) on three days of the week, for periods of 2 weeks, and 1, 2, 3, 10, 11, 13 and 14 months. The animals were 6 to 8 weeks old at the beginning of the experiment. At autopsy the bones were removed and subsequently prepared for microscopic study in the same manner as in our former investigations (1941a).

MICROSCOPIC EXAMINATION

(1) *Zone of endochondral ossification*:

After two weeks of administration of thyroxin, the epiphyseal disk of the upper tibia was enlarged. Whereas, in untreated animals of

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corresponding strain and age (Fig. 1)*, the length of the cartilage cell rows begins to decrease and greater amounts of sclerosed matrix cause the columnar arrangement of the growth zones to become more irregular, in mice injected with thyroxin (Fig. 2) the cartilage cell rows were high and contained many cells. The columnar structure of the cartilage cell rows was still quite distinct, the ground substance was scarce and chondromucoid in character. The non-oriented cartilage cells were spindle-shaped and showed long plasmatic processes. In a single cartilage cell row 10 to 11 columnar cartilage cells were counted. instead of 7 to 8 as is normal for this age. These cells proliferated markedly, and frequently by way of mitotic division. They were conspicuously enlarged, took on a polyhedric and ellipsoid shape, and their cytoplasm became slightly honey-combed. The conversion of these cells into cells of hypertrophic type began to take place more proximally in the epiphyseal zone than ordinarily. However, even in the most distal parts of the epiphyseal disk they were not quite as large as the largest hypertrophic cartilage cells under normal conditions. One has the impression that the processes of both hypertrophy and breakdown of the cartilage cells were so greatly accelerated that, on the one hand, the majority of the columnar cells became hypertrophied, and, on the other hand, the hypertrophic cells broke down before they could reach their maximum size.

After continuation of the injections for a period of one month, the zone of endochondral ossification was greatly narrowed (Fig. 3). The cartilaginous matrix had increased in amount and density. More calcium salts than usual were deposited in the most distal layers of the cartilage cells, and not infrequently the cells became enclosed by acidophilic, pre-osseous ground substance and thus were converted directly into osteocytes. The cartilage columns were rather irregularly arranged and were shorter and less numerous than normally. An exact cell count could no longer be made. Simultaneously, atrophy and degenerative processes had affected individual cells as well as whole cell rows, and had converted them into amorphous material. In some of these areas, hyalinization and ossification had set in and led to the formation of numerous fairly thick bony plugs, traversing the entire width of the cartilaginous plate from the metaphysis into the epiphysis. The condition in the epiphyseal disk of these mice at the age of 10 to 12 weeks was comparable to that of normal mice of the corresponding strain 4 to 6 months of age (Silberberg and Silberberg, 1941a).

These changes became still more accentuated if thyroxin was given for periods of two months and over. After two months of injections, matrix and cells of the epiphyseal cartilage were even more intensely

*We are indebted to Mr. S. J. Hayward for the Photomicrographs.

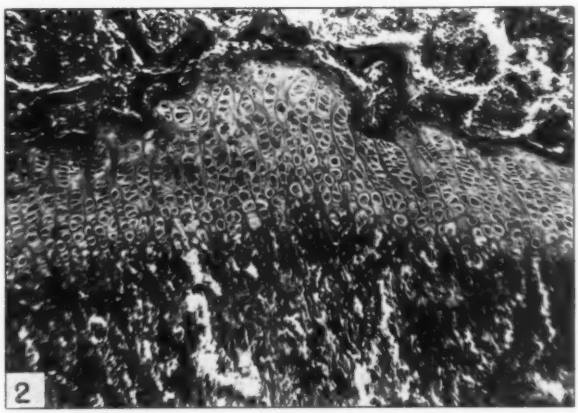
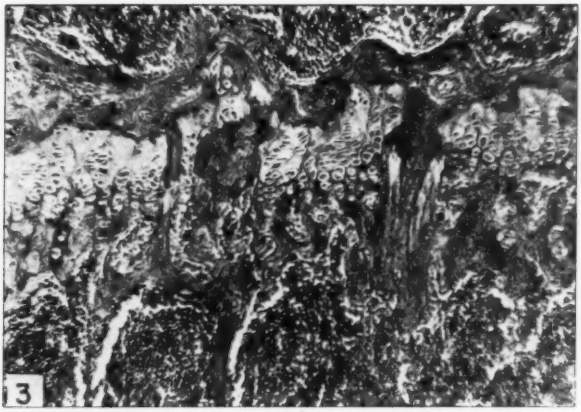


Fig. 1. Section through the epiphyseal zone of the upper tibia of a normal female D mouse two months of age. Magnification 150×.

Fig. 2. Section through the epiphyseal plate of the upper tibia of a female D mouse two months of age, which starting at the age of six weeks, had received injections of 0.1 mg of thyroxin three times weekly. Compare with Fig. 1. Epiphyseal disk somewhat enlarged, cartilage cell rows higher, columnar cartilage cells enlarged, large hypertrophic cartilage cells lacking, cartilaginous ground substance scarce, bony trabeculae thin. Same magnification as in Fig. 1.



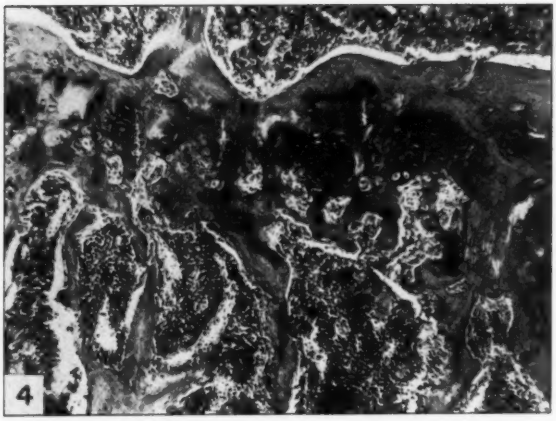


Fig. 3. Section through the epiphyseal zone of the upper tibia of a female D mouse two and a half months old, which had been injected with 0.1 mg thyroxin three times weekly for a period of one month. Epiphyseal zone narrowed and sclerosed, containing thick osseous plugs. In the subepiphyseal zone there is a beginning formation of a transverse bony lamella. Short and thin osseous spicules. Magnification 150×.

Fig. 4. Section through the epiphyseal zone of the upper tibia of a female D mouse three and a half months old, which, starting at the age of six weeks, had received injections of 0.1 mg thyroxin three times weekly. Compare with Fig. 3. The epiphyseal zone is greatly narrowed,sclerosed, calcified and ossified. The subepiphyseal osseous plate and the bony trabeculae have become thickened. Same magnification as in Fig. 3.

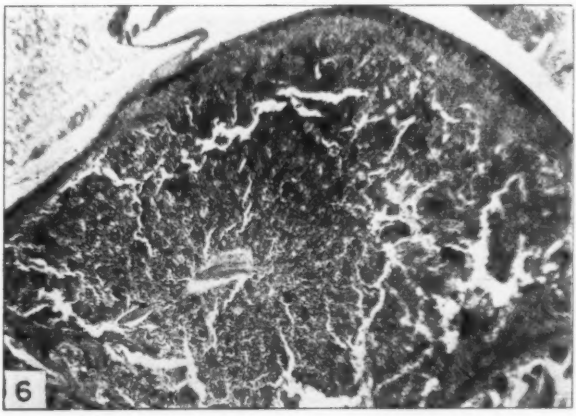


Fig. 5. Section through the epiphyseal plate of the lower femur of a female D mouse three and a half months old, which had been injected with 0.1 mg thyroxin three times weekly for a period of two months. Solution processes have led to a perforation of the epiphyseal plate. Conditions otherwise similar to those in No. 4. Same magnification as in Fig. 4.

Fig. 6. Section through the epiphyseal zone of the upper tibia of a female D mouse thirteen months old, which starting at the age of two months, had received injections of 0.1 mg thyroxin three times weekly. Complete epiphyseo-diaphyseal union has taken place. Two small remnants of bone indicate the site of the former epiphyseal plate. Magnification 120×.

calcified, sclerosed and ossified (Fig. 4). Frequently, owing to dense incrustation with calcium salts, details in the structure of the cartilage cells could no longer be detected. Centers of retrogression and ossification had become more numerous and more extensive. At the same time, capillaries of the bone marrow advanced towards and into the osseous plugs, and on account of capillary absorption and increased osteoclastic activity, the bony structures were at first perforated and later dissolved, producing increasingly widening gaps in the epiphyseal plate (Fig. 5). Thus, under the influence of thyroxin, in D mice, epiphyseo-diaphyseal union was in progress at the age of three and a half months, a condition found in non-injected mice of this strain not before the age of six to seven months. With extension of the duration of the experiment, degeneration and ossification of the epiphyseal cartilage, as well as solution of the almost completley ossified epiphyseal plate, made further advance.

After ten or more months of injections, the epiphyseal plate had been entirely dissolved (Fig. 6), and a complete epiphyseo-diaphyseal union had taken place. Only remnants of a narrow bony lamella arranged in a transverse direction indicated the site of the former epiphyseal zone. A similar stage of epiphyseo-diaphyseal union has not been observed in normal mice of any strain or age. The changes in the distal epiphyseal zone of the femur took a course similar to that seen in the proximal part of the tibia.

In the three mice belonging to strains C57 and Old Buffalo the reaction of cartilage and bone was the same in kind as in the mice of strain D. However, the processes were somewhat delayed as compared with the corresponding stages in mice belonging to strain D.

(2) *Subepiphyseal zone*:

After two weeks of injections of thyroxin, the subepiphyseal layer contained congested capillaries surrounded by closely packed connective tissue cells. These cells proliferated mitotically and were quickly converted into epithelioid osteoblasts. The osseous ground substance deposited was dense but not large in amount, and consequently the bony spicules were thin. Osteoclasis was marked and led to a shortening and thinning of the trabeculae.

After thyroxin had been administered for one month, the number of spicules was decreased because of the continued resorptive processes. However, the trabeculae which were still present were fairly long and thickened as compared with the earlier stage. Underneath the epiphyseal disk a transverse osseous plate had begun to form, which latter is normally only seen at the end of the fourth month.

After thyroxin had been injected for two or more months, the bony

trabeculae became shorter and the transverse osseous plate was more and more perforated by capillaries. After nine months or more of treatment, the subepiphyseal bony structures had been completely dissolved and longitudinal spicules were no longer seen.

(3) *Joints*:

In the articular cartilage the early changes following the administration of thyroxin consisted of an intensification of the growth processes. The cartilaginous ground substance was diminished. The cells of the uppermost, the sliding zone, and of the underlying transitional zone increased in number; the cells of the transitional zone arranged themselves in a perpendicular direction and formed short cartilage cell columns. The bony border lamella which delimits the hypertrophic cartilage cells from the bone marrow was thinned out. In some places, congested capillaries and multinucleated osteoclasts absorbed the osseous border lamella and elements of the bone marrow penetrated into the cartilaginous covering. After one month of injections, the proliferation of the cells of the various layers of the covering of the joint became less accentuated, whereas hypertrophic changes became pronounced; the resorptive processes made progress and the amount of bony tissue present was still further diminished.

After two months of treatment, the hyperplastic and hypertrophic as well as the resorptive processes receded, and, at still later periods, the opposite condition was observed. After three or four months, the cells had become even smaller than normal and a dense osseous tissue had replaced the hypertrophic and the deeper layer of the transitional zones.

After nine months and later, a slight increase in the amount of bone could be noted, but the total amount of bone formed was less than usual. Otherwise no changes were observed as compared with the earlier stages. Under normal conditions, however, towards the end of the first and during the second year of life, proliferative and retrogressive changes occur which, in many instances, lead to arthropathic lesions of varying degree, particularly in mice belonging to high tumor strains. Under the influence of prolonged administration of thyroxin, on the other hand, these age changes, if present at all, were less frequent and less severe than in non-injected animals. Of seven D mice over six months of age which had been injected with thyroxin, only one (14.3%) showed arthropathic changes, whereas in non-treated D mice of the same age the incidence of such changes had been found by the authors (1941a) to be 70%.

(4) *The bony shaft*:

Two weeks after beginning of the injections, an increased mitotic proliferation of the connective tissue cells was seen in the loosened

periosteal tissue. Some of these cells became converted into epithelioid cells, many of which, in contact with the bone, were transformed into osteoblasts, whereas others coalesced and formed multinucleated osteoclastic giant cells situated in grooves of the softened compact bone. The vascular canals of the bony cortex were enlarged.

After one or more months, the periosteal tissue and the compact bone became denser, the vascular canals narrowed and the resorptive processes were somewhat less accentuated, but the shaft remained thin, even at late stages of the experiment.

The bone marrow was cellular throughout the experimental period. In animals in the second year of life there was a tendency of the bone marrow to transform into fibrous tissue.

DISCUSSION

The effect of thyroxin on the epiphyseal growth zones consists in an intensification of the progressive and retrogressive processes involved in growth, differentiation and ageing of this tissue. Proliferation of the cartilage and conversion of the columnar into hypertrophic cartilage cells proceed at an increased rate at the early stages of the experiment. This intensification of progressive changes is followed by a likewise markedly accelerated and accentuated degeneration and ossification of the epiphyseal cartilage. Ossification may then take place directly and without a preceding destruction of the hypertrophic cartilage cells by capillaries of the bone marrow. Finally, there is also an increase in the solution processes, leading to a complete absorption of the epiphyseal plate and thus to a state of skeletal differentiation, which does not occur under normal conditions even in very old mice. Therefore, the possibility may be suggested that the failure or incompleteness of epiphyseo-diaphyseal union as seen in certain rodents, may perhaps be due to a lack of thyroid activity.

The changes in the growth zones of mice called forth by thyroxin resemble thus in some respects those which we observed after injections of bovine anterior hypophyseal extract (1941b). However, under the influence of thyroxin, both proliferative and retrogressive changes proceed at a much faster rate than under the influence of anterior pituitary extract, and the degree of epiphyseo-diaphyseal union is even more complete in the former than in the latter case. A further difference in the effects of these hormones seems to exist in that after administration of thyroxin the absorption of bony tissue is greater than under normal conditions, whereas in mice injected with anterior pituitary extract the apposition of bony tissue may be temporarily increased over normal. Similar findings have been recorded by Dott (1923) in dogs treated with thyroid and anterior pituitary hormone. This investigator observed that although skeletal growth and differentiation were equal-

ly hastened after administration of anterior pituitary hormone, in hyperthyroid animals skeletal growth was less accelerated than skeletal differentiation. Coryn (1939), however, who injected thyroxin into one young rabbit for a period of thirty days, believes that the increased growth taking place subsequent to the administration of thyroid hormone, is at a later date compensated by a premature cessation of the growth of cartilage.

The articular cartilage undergoes, under the influence of thyroxin, similar changes as the epiphyseal cartilage. But, whereas the alterations in the epiphyseal cartilage may be considered as an intensification of normal processes, a similar interpretation cannot be made as to the changes in the joints. In normal old mice we have frequently seen arthropathic lesions of varying degrees; they were severe and quite common (70%) in mice belonging to strain D. The incidence of these articular lesions in mice treated with thyroxin is so strikingly reduced (14.3%) that considerable importance must be attached to this effect. If thyroxin has the tendency to accelerate the ageing of the skeletal tissues, one should expect the age changes in the joints likewise to be intensified. Since this is not the case, and, on the contrary, even a decrease in the incidence of the arthropathic lesions is observable, it may be assumed that either thyroxin exerts a special effect on the tissues of the joint, or that the arthropathic lesions seen in normal mice are not due to old age as such, but that they are called forth by an endocrine or a metabolic disturbance, which merely coincides with old age and which may be counteracted by thyroxin. In this respect, also, the effect of thyroxin is different from that of anterior pituitary extract, under the influence of which the frequency and intensity of the articular lesions in older mice are increased.

The effect of thyroxin on the skeletal tissues of mice is thus similar to that on cartilage and bone of immature guinea pigs, which we observed previously, although the latter experiments extended over a period of only thirty days. During this short time, the increase in proliferation and the acceleration of differentiation of the epiphyseal cartilage and of the cartilage of the joints could be demonstrated, but the stage of accelerated degeneration and resorption of the epiphyseal cartilage cells had apparently not been reached at the conclusion of the experiment.

Stefanescu (1926), on the other hand, reported in growing guinea pigs, and Smith and McLean (1938) in growing rats, a decrease in the growth of the cartilage following a prolonged administration of thyroid hormone, but they did not investigate the early changes called forth by the hormone.

It might also be mentioned that the mice used in the present investigation were comparatively older than the guinea pigs formerly used and that the amount of thyroid hormone given to the mice was relatively much greater than that administered to the guinea pigs. It is conceivable that, under the influence of the large dose, the changes produced by the hormone proceed at a faster rate. Likewise, the greater age of the mice at the begining of the treatment might be responsible for the increased tendency of the cartilage to degenerate and its decreased tendency to proliferate under the influence of thyroxin, an effect which we (1939) were able to demonstrate in the case of other hormones acting on the cartilage.

SUMMARY

In growing mice, administration of thyroxin accelerates first proliferation, then ossification, and in the end resorption of the epiphyseal cartilage and of the cartilage of the joints. Resorptive processes are not only accelerated but also increased, and thus lead to a complete epiphyseo-diaphyseal union, which does not take place in normal mice of corresponding strains at any age. In general, thyroxin accelerates and intensifies therefore the age changes in the skeleton; however, it causes a decrease in the severity and incidence of certain old age changes which occur in the joints of normal mice.

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STUDIES ON FRESH-WATER BRYOZOA

XI. THE VIABILITY OF DRIED STATOBLASTS OF SEVERAL SPECIES

By

MARY D. ROGICK

College of New Rochelle

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The present study gives further information on the viability of dried statoblasts of *Lophopodella carteri* var. *typica* (previously discussed in Study VII) and also some notes on the viability of dried statoblasts of *Pectinatella magnifica, Fredericella sultana, Hyalinella punctata, Plumatella repens* var. *typica* (*beta* phase) and *Plumatella repens* var (?). Data concerning collection sites, number of days of drying, etc., are to be found in Table I.

The *Lophopodella* specimens were collected on August 25, 1933. Living colonies of this species were brought into the laboratory. Many were loaded with developing statoblasts. Later some of the colonies were allowed to degenerate and disintegrate in finger bowls and crystallization dishes until the statoblasts were released. Other colonies were deliberately torn apart to permit the release of statoblasts. All these statoblasts were kept in a large crystallization dish containing some plant material, snails and greenhouse tank water until November 6, 1933. Then they were taken out, placed on a sheet of hard-surface drawing paper and allowed to dry on a sunny window ledge in a warm room. After several days, when they appeared to be thoroughly dry, the statoblasts were scraped off the paper very carefully and placed in a large glass vial, where they remained uncorked for several days. After corking the vial it was put away in a box and kept in darkness (closet or desk) at room temperature until needed. At the end of various intervals (see Study VII and present study, Table I) some statoblasts were taken out and inspected for "blanks" (immature, already hatched or deformed statoblasts). The abnormal ones were generally discarded and only mature, "good" statoblasts used for the experiment.

Hyalinella and the two *Plumatellae* were dried in a similar manner, except that they were not kept for a long interval in fresh water until the colonies degenerated or disintegrated, as were the *Lophopodella* specimens. Rather, the *Hyalinella* and *Plumatella* specimens were kept for a week or two (depending upon the specific experiment) in the water before being dried. They were dried exactly as were the *Lophopodella* specimens and cared for subsequently in the same manner. They were somewhat harder to handle than *Lophopodella* statoblasts because of their smaller size.

TABLE I

ESSENTIAL DATA ON ALL THE SPECIMENS USED IN THE PRESENT STUDY

Species	Duration of drying	Number of statoblasts		Temperature maintained, in degrees Centigrade			Collected at
		Tested	Germinated	Average	Highest	Lowest	
Lophopodella carteri var. *typica*	a. 1543 days = 50.7 months	10	7 (see Table	26.3	30.0	23.0	East Harbor in Lake Erie, Ohio
	b. 2252 " = 74.0 "	10	0 II for	25.3	32.0	18.0	
	c. 2278 " = 74.8 "	15	0 more data)	24.9	31.0	20.2	
	d. 2416 " = 79.4 "	25	0	26.3	34.0	21.0	
	e. 2478 " = 81.4 "	50	0	24.6	26.0	23.0	
Hyalinella punctata	a. 1072 " = 34.9 "	26	0	27.4	32.0	21.0	Beechmont Lake in New Rochelle, New York
	b. 1094 " = 36— "	26	0	24.6	26.0	23.0	
Pectinatella magnifica	a. 1052(?)* = 34.5(?)*	12*	0*	26.3	34.0	21.0	a. Lake Stahahe near Southfields, N. Y.*
	b. 1737 days = 57.1 months	121	0	26.3	34.0	21.0	b. and c. Paine Lake in New Rochelle, N. Y.
	c. 1757 " = 57.7 "	100	0	26.9	32.0	21.0	
Fredericella sultana	a. 731 " = 24.0 "	12	11 (see Table	27.4	30.0	24.8	Fresh Water Pond, Woods Hole, Mass.
	b. 752 " = 24.6 "	7	7 III for	26.3	28.0	25.0	
	c. 765 " = 25.1 "	8	4 more data)	24.6	26.0	23.0	
Plumatella repens var. (?)	a. 2555 " = 84— "	50	0	26.9	32.0	21.0	Pelee Island in Lake Erie, Canada
	b. 2597 " = 85.2 "	24	0	24.6	26.0	23.0	
Plumatella repens var. *typica*	a. 704 " = 23.1 "	100	0	24.6	26.0	23.0	a. Huguenot Lake in New Rochelle, N. Y.
	b. 777 " = 25.5— "	23	0	24.6	26.0	23.0	b. Gansett Pond in Woods Hole, Mass.

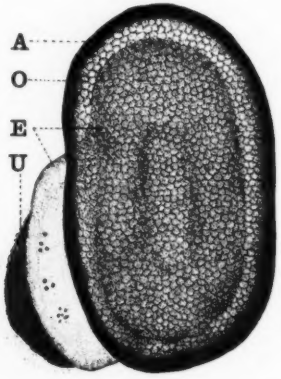
Explanation of Table I:

A dash (—) after a number in the above Table signifies that the time is only two days short of the number indicated.

An asterisk (*) denotes the specimens of *P. magnifica* which were collected by Mr. Edwin Newman.

The letters (a, b, c, d, e) refer to separate sets of experiments with the various species.

Three statoblasts of *Lophopodella carteri* (experiment *a*) were lost while all the remaining seven germinated.



A camera lucida sketch of a germinating *Fredericella sultana* statoblast. For further data on this please refer to Tables I and III. The statoblast is mature as can be inferred from the dark, roughened, or mammillate appearance of the valve. The labels refer to the following structures:—*A*—capsule or valve, *E*—germinative material, *O*—mammillations, *U*—yolk material. A 12.5 × ocular and a 10 × objective were used in this sketch.

Fredericella statoblasts differed from those of the other species in that they were sessile or fixed (attached to the zooecial tubes), hence had to be handled somewhat differently. The removal of each individual *Fredericella* statoblast was too tedious a task, so zooecial tubes were torn apart in places, for quick drying, and the zooecial fragments which contained the statoblasts were dried in the open on paper, then when dry placed in vials and stored. When needed for the experiment these fragments of colonies were removed from the vial, very gently rubbed between the fingers until the colony fragments were reduced to a powder. The statoblasts which adhered to the zooecial walls were thus freed from them without apparent damage. If any zooecial fragments debris were adhering to the statoblasts of the other spineless species (*Plumatella* or *Hyalinella*) these could be removed reasonably well by the same treatment. Because of the spines or processes on the *Pectinatella* and *Lophopodella* statoblasts rubbing to clean them of adherent material was not feasible since the spines might be broken off. Some of the *Pectinatella* statoblasts were dried in the same manner as the *Lophopodella* statoblasts but most of them were placed on a clean slide (50 or 100 statoblasts per slide) and allowed to dry there, then when dry stored away in a wooden slide box. This was the most convenient method of handling statoblasts. When desired for an experiment a slide to which the *Pectinatella* statoblasts were adhering could be immersed in water in a Petri dish. In a few days the statoblasts would be soaked loose from the slide, then could be transferred to syracuse watch glasses. The statoblasts which had been attached to the slide were found to have a much better appearance than those which had been dried in the ordinary manner in dishes or on paper, unattached to slides. The capsules looked more plump and the processes were in better condition.

Many of the statoblasts of the different species, especially of the *Plumatellae*, after some time in the dried state, showed a "collapsed" capsule, i. e., a capsule one of whose sides was slightly sunken in, instead of being gently curved outward. However, after these statoblasts were immersed in water the natural shape was restored to a fair extent. Some of the *P. repens typica* statoblasts, undamaged as far as could be seen, had one or two air bubbles within the capsule in the germinative material. When these bodies were immersed the air bubbles were replaced in a minute or two by water. A light patch was then evident within the capsule where the air had been. If considerable pressure is exerted on a statoblast which has these air bubbles the capsule can be cracked and the white, concentrated, rather dry-looking germinative material can be forced out.

Another characteristic of statoblasts which had been dried for a long

time is the emptier, more translucent and lighter colored appearance of the capsule.

Dried statoblasts of the various species were placed in separate syracuse watch glasses to which tap water was added at first. As the water slowly evaporated the original level was maintained by small additions of aquarium water so that in time the statoblasts were in aquarium water entirely. The water in the dishes was changed occasionally when necessary, aquarium water being used. In the case of *Fredericella* which germinated very rapidly the tap water was replaced by the aquarium water at the first sign of germination. Among the organisms present in the aquarium water were the following:—*Actinophrys sol, Amoeba radiosa, Colpidium, Lacrymaria, Paramecium, Stentor,* suctorians, oligochaetes, rotifers and algae. When water was first added to the statoblasts most of the dried statoblasts rose with the water level and floated to the surface. These were then pushed down into the water with a dissecting needle so that they would become thoroughly immersed or wet. Some sank immediately to the bottom of the dish while others, being more buoyant, bounced back again to the surface. In the case of *Lophopodella* the undamaged ones bounced back again to the surface or to the edge of the dish within the surface film. On a number of occasions they had to be pushed back into the water because as the water evaporated they would be left stranded along the edges of the dish, dry, above the water level. The *Plumatella* and *Hyalinella* statoblasts behaved similarly. *Pectinatella* and *Fredericella* statoblasts however, when thoroughly wet, sank down to the bottom of the dish. Daily examinations of the dishes had to be made to prevent statoblasts from getting out of the water and to keep close watch on any germinations.

The *Lophopodella* specimens were from the very same source and collection as those discussed in Study VII on p. 184 and were taken from the glass vial in which they had been kept since 1933 at various intervals (1543, 2252, 2278, 2416 and 2478 days respectively). They were decorated with a number of barbed spines or processes at each pole, as is characteristic for this species. Each process is double in nature as shown in Study IV. In most instances these processes were partially split or separated into their component halves at the time of their immersion. In some cases they were not separated at the start. In those statoblasts which germinate, the splitting proceeds to the stage where the valves completely separate from each other. *Lophopodella carteri* statoblasts proved viable and capable of germinating and evaginating in a short time after $4\frac{1}{4}$ years of drying. Those which had been dried longer than 6 years failed to germinate. The viability record for this species' statoblasts appears to be much better than that for the other species studied.

When the *Lophopodella* statoblasts first germinated the yolk material was found in various places. Some of it was in the basal part of the colony, lining the body wall, some stuck to the intestine and some occurred in the lophophore, at the base of the tentacles and also in the lumen of the tentacles. It disappeared as the polypides increased in size and vigor. Because of the scarcity of food the *Lophopodella* colonies did not develop very far, only to 1 or 2 polypides in this latest series of experiments (please see Table II), although there were a number of buds or potential polypides. When degeneration of a colony set in the polypides disappeared in the usual manner (Study VII, p. 197) and the colony was reduced to a mere bag of tissue. These sacs remained alive for a number of days, some for as long as 24 days. One could tell that they were alive by their general appearance and also because occasionally they would alter their shape very slightly. They were thinwalled, quite transparent and light in color.

TABLE II

GERMINATION DATA ON *Lophopodella carteri* VAR. *typica* STATOBLASTS WHICH HAD BEEN DRIED FOR 1543 DAYS AND IMMERSED IN WATER ON JANUARY 27, 1938

Specific number of the statoblast	98	99	100	101	102	103	104
Number of days between immersion and germination	7	12	18	19	19	20	20
Number of days between immersion and evagination of first polypide	8	13	20	21	21	22	22
Number of days between germination and evagination of first polypide	1	1	2	2	2	2	2
Number of days between germination and evagination of second polypide	5						
Number of days from germination to death or loss of colony	38	33	8*	26	26	31**	31**
Total number of polypides evaginated	2	1	1	1	1	1	1

*Dried out accidentally.

**Still a living sac with some degenerating material at the time of the termination of the experiment summarized in the above Table. The food problem accounted for the low figure of numbers of polypides.

The polypides were not at all averse to ingesting or "swallowing" rotifers. When these organisms were taken into the stomach they remained very active, coming up continually against the esophageal valve. After about 10 minutes some of these rotifers were transferred by the polypide from the stomach to the rectum and remained in the latter

place for at least 20 minutes longer, still alive and moderately active. They were eliminated from the digestive tract of the polypide in a pellet of waste material. In time they worked themselves free of the pellets and proceeded to swim about as if nothing had happened to them.

Of the other species, the *Fredericella sultana* statoblasts were the only ones to germinate after a very long drying period. The *Fredericella* statoblasts which had been dried for slightly more than 2 years germinated in a very short time (refer to Table III) but failed to

TABLE III

GERMINATION DATA ON *Fredericella sultana* STATOBLASTS WHICH HAD BEEN COLLECTED ON JULY 9, 1938 AND DRIED SINCE JULY 15, 1938 (FOR ADDITIONAL DATA PLEASE REFER TO TABLE I)

Statoblast number	Date immersed	Number of days between immersion and germination
120, 121, 122	VII- 16- 1940	1
123, 124, 125, 126	" " "	2
127, 128, 129	" " "	3
130, 131	" " "	7
132, 133, 134, 135 136, 137, 138	VIII- 5- 1940	2/3 of a day
139	VIII- 18- 1940	1/4 day
140	" " "	5/6 of a day
141, 142	" " "	1 3/8 days

evaginate. Some of these germiated in as short a time as 6 hours after immersion in water. The first indication of germination was a faint, very delicate fuzz which appeared in the region where later the germinative material would protrude between the valves. The first part of the germinative material to protrude was a small cresent of opaque substance (see Fig. 1) which resembles or probably corresponds to the yolk material of *Lophopodella*. This material disappeared as the germinative material increased in size. The latter was translucent at first but later became a little more transparent. Degeneration set in shortly, several days after germination.

Unfortunately, I did not attempt to germinate any *Pectinatella magnifica* statoblasts which had been dried less than $34\frac{1}{2}$ months. The data in this paper is for *Pectinatella* statoblasts which had been dried $34\frac{1}{2}$ and 57^{+} months (refer to Table I). No germinations were obtained from these. This was in line with Dr. Brown's (1933, p. 312) results. He failed to obtain germinations from *Pectinatella* statoblasts which he had dried for about 4 months (120 days). However he did obtain a 24% germination (p. 301) from those statoblasts which had been frozen for al-

most 10 months (300 days). As far as the general appearance of the New Rochelle specimens was concerned, it was very good although the statoblasts had been dried for several years. Apparently the *Pectinatella* statoblasts have not the same resistance to long periods of drying as do the *Lophopodella* statoblasts.

The *Hyalinella* and *Plumatella* specimens failed to germinate after being dried for a number of months (for exact length of drying period, please see Table I) hence one must conclude that drying for a considerable length of time is fatal to these lighter and more delicate statoblasts and that their viability period is shorter than that for dried *Lophopodella* statoblasts. Braem (1890, p. 89) stated that Trembley had obtained germinations from dried *Plumatella* statoblasts which had been stored away from Sept. 1745 to Jan. 1746.

SUMMARY

There was considerable difference among the statoblasts of the several species in their ability to remain viable after long periods of drying.

The germination results from statoblasts dried considerable lengths of time proved negative in the case of *Pectinatella magnifica*, *Hyalinella punctata*, *Plumatella repens* var. *typica* and *P. repens* var. The first species was dried from 34.5 to 57.7 months, the second from 34.9 to almost 36 months, the third from 23 to 25.5 months and the fourth species from 84 to 85+ months.

Positive germinations were obtained from long-time dried statoblasts of *Lophopodella carteri* var. *typica* and *Fredericella sultana*. The results obtained from the *Fredericella* statoblasts which had been dried from 24 to 25.1 months were not so satisfactory as those from *Lophopodella*. The dried *Fredericella* specimens did however germinate very speedily after immersion but evagination of polypides failed to occur. Degeneration took place in a few days.

Lophopodella statoblasts which had been dried 50.7 months (almost $4\frac{1}{4}$ years) germinated, their polypides evaginated and the colonies remained alive for a number of days, but those statoblasts which had been dried from 74 to 81.4 months (more than 6 years) failed to germinate. As far as the writer knows this record of *Lophopodella* germination after $4\frac{1}{4}$ years of drying is much longer than for any other species of fresh-water Bryozoa which have been experimented with. *Lophopodella* seems to lend itself very well to experimentation of this sort because of the size and hardiness of its statoblasts.

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